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Partial-Trisomy Syndromes I. Sturge-Weber's Disease*

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INTRODUCTION

To DATE three autosomal trisomy syndromes are known in man; that is there are at least three autosomes each of which may be present in triplicate without necessarily having a lethal effect. Each of the three types of trisomy interferes in a specific and serious manner with development, all of the resulting syndromes involving mental retardation. The three syndromes are: mongolism, the No. 18 trisomy syndrome, and the D_1 syndrome (for references see Therman, Patau, Smith, and DeMars, 1961).

Hayward and Bower (1960) seemed to have found a fourth type of autosomal trisomy when they described a boy with the syndrome of Sturge-Weber who had 47 chromosomes, apparently being trisomic for a G chromosome (G is the symbol for the small acrocentric chromosomes, Nos. 21 and 22, in the classification by Patau, 1960). If so, the extra chromosome would obviously be the G autosome that is not responsible for mongolism. However, a single case does not suffice to establish trisomy if this word is taken to imply, as it should, the presence of three fully homologous chromosomes. An extra chromosome could also be a translocation chromosome.

Upon learning of Hayward and Bower's discovery we began investigating the chromosomes of patients with Sturge-Weber's syndrome in order to test the assumption of trisomy. It was not confirmed: in all of the three cases examined we found 46 chromosomes. The same has since been reported for a total of 14 individuals with this disease (Lehman and Forssman, 1960; Gustavson and Höök, 1961; Hall, 1961; Hayward and Bower, 1961). Whereas in these 14 and in two of our cases no chromosomal abnormality was detected, the third of our patients did have a translocation. This corroborated Hayward and Bower's original result to the extent that a chromosomal abnormality of some kind is at least sometimes present in Sturge-Weber patients. We believe it is always present, even though its microscopic demonstration may usually be impossible with the available methods. In the discussion, we shall advance an interpretation in which the Sturge-Weber syndrome appears as a prototype of a large, etiologi-

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FIG. 1. The three patients. From left to right: Cases 117, 141, and 146.

cally unique, class of syndromes, individually rare but collectively probably accounting for a substantial part of all multiple congenital anomalies including those with mental retardation.

THE PATIENTS

The clinical evidence need be presented only to the extent necessary to establish that all three patients have Sturge-Weber's syndrome according to the criteria listed by Greenwald and Koota (1936). All three are Caucasian children who display severe mental retardation, seizures, and congenital cutaneous angiomatous nevi involving the face and other areas of the body (Fig. 1). In addition, intracranial calcification was demonstrated by roentgenograms in the Cases 117 and 146, females of six and fourteen years of age, respectively. In Case 141, a five year old male, intracranial angiomatosis was revealed by surgical exploration as well as by X-rays.

All three patients have two or three apparently normal sibs, and there is no family history of similar afflictions. At the time of birth of the patients the maternal ages were 22, 25 and 28; the paternal ages 25, 31 and 33. None of the parents is known to have been exposed to excessive radiation.

BLOOD CULTURE AND CYTOLOGICAL METHODS

Leukocytes from peripheral blood were cultured by Nowell's method following a procedure that differed only in minor respects from that described by Moorhead, Nowell, Mellman, Battips and Hungerford (1960). It was found that blood in a sterile sealed container can be transported on ice for more than five hours without apparent detriment to the subsequent culture. If the blood is kept in a refrigerator (5° C), even a delay of 24 hours seems to do little harm. Colchicine was applied, on the third or fourth day of culture, in a final concentration of 0.0002% for only two hours of additional incubation. After centrifugation, the cells were resuspended in an 0.95% solution of sodium citrate in which they were left in the incubator for 25 minutes. After another centrifugation the

TABLE 1. CHROMOSOME COUNTS, NEAR-DIPLOID CELLS ONLY. CULTURED LEUKOCYTES

Case	44	45	46	47	48	Tot.
117	—	—	9	—	—	9
141	1	—	30	—	—	31
146	—	1	18	—	—	19
Total	1	1	57	—	—	59

material was fixed in 1:3 acetic alcohol and later transferred into 70% alcohol for storage.

While we grant that the air-drying method can yield very good slides, it seems to us that human chromosomes are rendered somewhat more distinct if drying is avoided. Feulgen staining was used throughout and as a rule followed by a moderate application of acetic orcein, a combination that yields well stained chromosomes, satisfactory for showing satellites and for photographic purposes, and yet leaves the plasma unstained and the cells soft enough for any desired degree of squashing. This was done in 50% acetic acid, the slide having been made semi-permanent by sealing with Kroenig cement.

CYTOLOGICAL RESULTS

All patients have 46 chromosomes (table 1). Although there was relatively little material from Case 117 it sufficed for a detailed analysis of all chromosome groups. The preparations obtained from the other two patients were still more satisfactory. The patients 117 and 146 have chromosome complements in which a diligent search failed to reveal any abnormality (Fig. 2).

In Case 141 all chromosome groups seem to be normal with the exception of the D group, which is lacking one chromosome. Instead, there is a chromosome

FIG. 2. The 46 chromosomes of Cases 117 (left) and 146 (right).

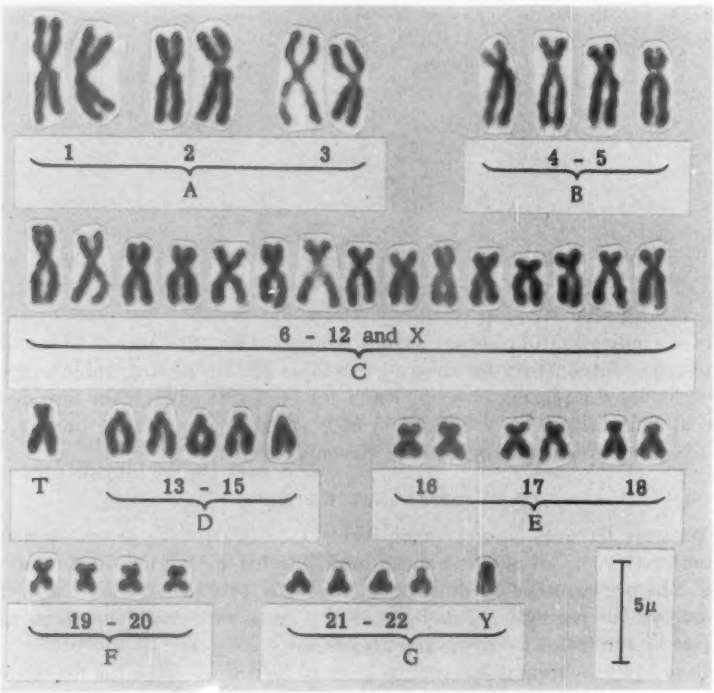

FIG. 3. Karyotype of Case 141. T: translocation chromosome. Classification according to Patau (1960); see also Patau (1961).

that resembles a D chromosome in its long arm but has too large a short arm (Figs. 3 and 4). On the other hand, this short arm appears to be a little smaller than that of any C chromosome, which would rule out trisomy for one of the latter. However, it would be rather absurd anyway to assume that the patient is trisomic for a C and monosomic for a D chromosome. It can be considered as certain that the chromosome in question represents a D chromosome to which a segment of an unknown donor chromosome had been attached by translocation. The short arm of the translocation chromosome seems to be slightly smaller than the long arm of No. 22.

DISCUSSION

To our knowledge, 18 Sturge-Weber patients have been investigated cytologically. The results were: one case with 47 chromosomes (Hayward and Bower, 1960, 1961); one, our Case 141, with a translocation chromosome; our Cases 117 and 146 with seemingly normal complements; and 14 more cases for which, with the exception of the two that were insufficiently analyzed, other authors have reported the same lack of a recognizable abnormality (Lehman and

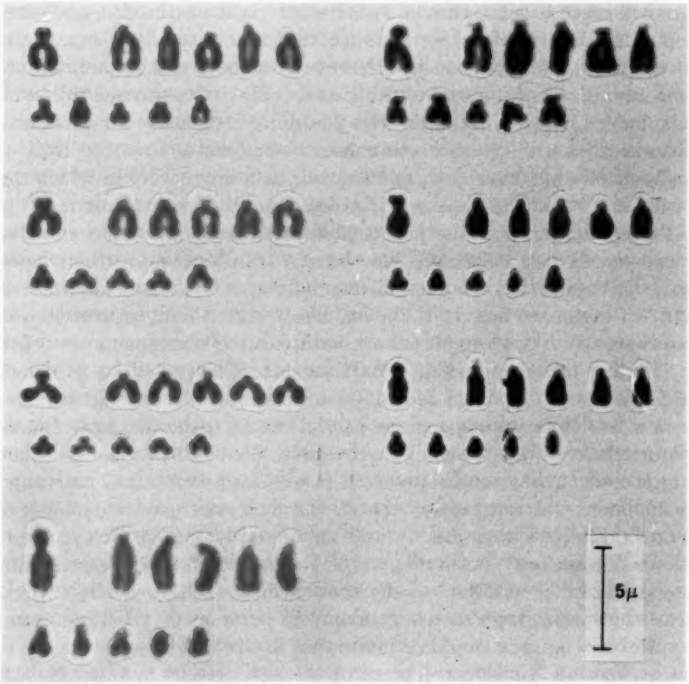

FIG. 4. Case 141. The translocation chromosome, five normal D chromosomes, and the G chromosomes of seven cells.

Forssman, 1960; Gustavson and Höök, 1961; Hall, 1961; Hayward and Bower, 1961).

A human complement with an extra chromosome, in particular if the sex chromosomes and the mongolism chromosome are excluded, is very rarely encountered. So is a detectable translocation in man. We may, therefore, take for granted that the co-occurrence of the very rare Sturge-Weber syndrome with either kind of chromosomal abnormality is not a mere coincidence. Concluding then that in both cases the extra chromosomal material has caused the syndrome, we may further take for granted that the extra chromosome in the case with 47 chromosomes and the translocated piece in Case 141 have a segment in common in which a gene or genes are located that cause the Sturge-Weber syndrome when present in triplicate.

The same chromosome segment might be present also in the other patients as part of a translocated extra piece. The Cases 117 and 146 definitely do not have the translocation chromosome of Case 141; but the human complement has many chromosomes, for instance those of the C group, in which a relatively small translocated piece could not be recognized in the microscope. Of course,

a priori it is also possible that in these other patients the Sturge-Weber syndrome had a different origin, for instance, that it resulted from a gene mutation or that it represented a phenocopy. However, one hesitates to contemplate more than one kind of causation for anything as specific and as rare as this syndrome. There is, indeed, no reason to take this possibility seriously, for we must expect that for every case in which a chromosome segment is involved in a microscopically detectable translocation there will be many others in which the same segment is involved in a translocation that cannot be thus recognized.

Much has been made of the fact that in practically all of the relatively few cases reported to date of persons who have a translocation in their basic complement, the breaks had occurred in the short arm or the centromere region of acrocentric chromosomes. It is indeed likely that these regions have a particular propensity to undergo breakage with ensuing rearrangement (cf. Polani, Briggs, Ford, Clarke and Berg, 1960; see also, for instance: Michaelis and Rieger (1958) who demonstrated in *Vicia* crowding of breakage and re-union points in a few chromosome regions partly known to be heterochromatic) but they cannot have a monopoly in this respect. We shall present elsewhere evidence to the effect that breaks involved in viable chromosomal rearrangements are by no means restricted to acrocentric chromosomes or to centromere regions. It should be realized how heavily and unavoidably biased all cytological observations on translocations in man are. A small translocated segment can easily be recognized if it is attached to the short arm of an acrocentric chromosome, but these short arms represent only about 11 per cent of all chromosome ends of the autosomal set. We would estimate that in the best of slides the percentage of ends at which a translocated piece of the size present in Case 141 could be detected lies somewhere between 16 and 32 per cent, but we suspect that the actual score would usually be lower, possibly much lower.

Furthermore, we have unpublished evidence indicating that in the basic complements of viable zygotes, insertions, which involve at least three breaks, are not as rare relative to the incidence of simple two-break translocations as one might expect. The recipient of an insertion is likely to be a large chromosome in which normally (i.e. in the absence of a special marker) a small insertion could not be discovered microscopically. Thus, there is additional reason to suspect that in a great majority of cases the presence of a small extra piece will remain undetected as long as we rely exclusively on cytological evidence. Therefore, the negative cytological result in 16 of 18 cases does not justify doubts about the Sturge-Weber syndrome being an etiological entity.

If the patient with 47 chromosomes were a trisomic, in other words, if Sturge-Weber's disease were primarily a trisomy syndrome, our Case 141, and probably also the other cases with 46 chromosomes, would be analogous to the exceptional mongoloids with 46 chromosomes who evidently have a major part of the mongolism chromosome in triplicate because of a translocation (Polani, Briggs, Ford, Clarke and Berg, 1960; Carter, Hamerton, Polani, Gunalp and Weller, 1960; Fraccaro, Kaijser and Lindsten, 1960; Penrose, Ellis and Delhanty, 1960; Buckton, Harnden, Baikie and Woods, 1961).

The interpretation of the Sturge-Weber syndrome as primarily a trisomy syndrome can for the time being not be disproved, but there is little to recommend it. If it were true, the extra chromosome would be the small acrocentric one that is not involved in mongolism. The two G chromosomes are so similar in size and shape as to preclude great differences in non-disjunction rate. Yet the incidence of the Sturge-Weber syndrome seems to be smaller than that of mongolism by one to two orders of magnitude. Whereas in the State of Wisconsin we could probably trace about a thousand mongoloids, we have so far learned of only seven persons, including the three presented in this study, who are afflicted with Sturge-Weber's syndrome. To make the discrepancy worse, none of our three cases had arisen by non-disjunction. Cytologically, the Sturge-Weber syndrome differs from mongolism in still another way. Whereas translocation cases are evidently infrequent among mongoloids, the investigation of Sturge-Weber patients has to date produced only one case of possible trisomy as compared with at least one but most likely 17 translocation cases. This seems to rule out what otherwise might be a possible explanation of the rarity of the Sturge-Weber syndrome: that Sturge-Weber trisomy is much more frequent than it seems to be but that this cytological abnormality does not usually manifest itself in the typical Sturge-Weber phenotype. One should certainly expect that such low penetrance would also prevail in translocation cases.

It appears then that a different interpretation is needed, and there seems to be only one, if the hypothesis of Sturge-Weber trisomy is to be upheld. One would have to assume not only that trisomy for this chromosome is ordinarily lethal, the case with 47 chromosomes being a rare exception, but also that the lethal effect depends on the presence in triplicate of a locus (or loci) that is not included in the observed and the assumed translocations. It will immediately become apparent that this interpretation, while rendering the hypothesis of Sturge-Weber trisomy tenable, also renders it unnecessary. Why should not the case with 47 chromosomes, too, have come about by a translocation? We propose that generally the Sturge-Weber syndrome is caused by the presence in triplicate of a certain segment from an unknown donor chromosome, a chromosome that as a whole could not be present in triplicate without having, always or at least as a rule, a lethal effect.

A chromosome segment that, by reciprocal translocation or insertion, has been added to the complement is commonly called a "duplication", even though in the case of a diploid complement the segment will be present in triplicate rather than in duplicate.[1] This is bound to puzzle non-geneticists, and since

[1] It should perhaps be stressed that we are here concerned only with extra pieces of a sufficient size to be microscopically detectable at metaphase (at least when the recipient is the short arm of an acrocentric chromosome). Such gross chromosomal rearrangements, involving fairly large numbers of genes, are in man probably always detrimental and would be more so in the homozygous state. They have, of course, been studied in Drosophila and other organisms but should not be confused with the much smaller "repeats" and the like that are seen in normal salivary gland chromosomes of Drosophila and interpreted as evidence of past duplications, steps as it were in the evolution towards greater genetic complexity.

many of these are now forced to pay some attention to human cytogenetics it is perhaps for once justified to give way to semantic considerations by adjusting the terminology. We suggest restricting "duplication" to the haplophase and to call the presence of an "extra segment" in the diplophase, in other words, the presence in triplicate of this segment "partial trisomy", an almost self-explanatory term. The above mentioned mongoloids with 46 chromosomes are in this sense "partial trisomics". Partial trisomy has its origin in chromosome breakage.

The above interpretation of the Sturge-Weber situation amounts to the proposition that this disease is always, or at least as a rule, the result of partial trisomy. We find this hypothesis attractive for the following reason. It appears *a priori* certain that there is a large class of rare syndromes that are caused by partial trisomy. There is little doubt that most human autosomes do not admit of viable trisomy. If this were not so, more than three trisomy syndromes should have been discovered by now. On the other hand, in practically all chromosomes there must be segments that may be present in triplicate without a lethal effect but not without causing a variety of congenital anomalies. Our knowledge of the actual occurrence of humans who carry chromosomal rearrangements is still meager, but even so it is becoming clear, as will be pointed out elsewhere, that their frequency, though small, is not negligible. Because of this, we look upon the Sturge-Weber syndrome as merely the first recognized example, a prototype as it were, of the postulated large class of rare syndromes caused by an almost inexhaustible variety of chromosomal rearrangements. We suspect that this class contains many, or even a majority, of the multiple congenital anomalies that are so common among the mentally retarded.

Such "partial-trisomy syndromes" as do not preclude reproduction of afflicted persons will be inherited as dominant mutations, but they will differ from the latter by the occasional occurrence of phenotypically normal carriers. Consider, for instance, the case of a reciprocal translocation taking place in a cell of a person's germ line. Among his or her offspring there may be children with an entirely normal complement, as well as children with 46 chromosomes that include the two translocation chromosomes. Unless mutation at a break locus or position effect complicate matters, these children will be phenotypically normal carriers. In the case of partially trisomic mongoloids, carriers, though of a somewhat different kind, have been found in two or three consecutive generations (Carter *et al.*, 1960; Penrose *et al.*, 1960).

If an F_1-zygote of a carrier, or of a person in whom the translocation had occurred in the first place, obtains 46 chromosomes that include the one or the other translocation chromosome, a lethal effect, i.e. an abortion, is to be expected unless the involved deletion is very small, in other words, unless one of the two components of the translocation chromosome is an almost complete normal chromosome. Thus, the birth of a partial trisomic with 46 chromosomes presupposes that one of the two breaks had occurred very close to the end of the "recipient chromosome". If this end is euchromatic, "very close" may mean a very few loci, whereas in the case of a distal heterochromatic region a relatively much longer deletion may be tolerated. It is to be expected that the non-

lethal deletion that is likely to be present in every partial trisomic with 46 chromosomes produces sometimes an anomaly, or anomalies, in addition to those caused by the partial trisomy.

If after a reciprocal translocation, or in a carrier, non-disjunction occurs, a partial trisomic with 47 chromosomes may be born. If the extra chromosome, although a translocation chromosome, should resemble a normal chromosome an investigator may be led to assume that he has found a new type of viable trisomy, and we suspect that this is what happened in the case of the Sturge-Weber patient with 47 chromosomes. The translocation chromosome of a partial trisomic with 47 chromosomes may contain extensive segments of two different chromosomes, the result being a combination of two partial-trisomy syndromes.

It appears more than likely that in most cases of partial trisomy with 47 chromosomes non-disjunction had been no mere coincidence but, rather, a consequence of the structural heterozygosity. There is no reason to think that non-disjunction of this kind depends to an appreciable degree on parental age. The probability of a cell having undergone chromosome breakage is likely to increase with age, but this effect could not possibly be of the magnitude of the maternal age effect in mongolism. Therefore, it is to be expected that the incidence of partial trisomics, be it with 46 or 47 chromosomes, has a much more normal parental age distribution than is found in mongolism. In our three Sturge-Weber cases the maternal age ranged from 22 to 28 years, in the case with 47 chromosomes it was 32. In view of the likelihood that partial trisomy plays an important role in the etiology of multiple congenital anomalies it is relevant to note that in a study on the incidence of congenital malformations mongolism stands alone by the magnitude of the maternal age effect (Böök, Fraccaro, Hagert, and Lindsten, 1958).

Assume a partial-trisomy syndrome, such as Sturge-Weber's disease, to be caused by the presence in triplicate of a terminal segment of a donor chromosome, that as a whole does not admit of viable trisomy. There must then be a "limiting" locus so that partial trisomy, too, becomes lethal once this locus is included in the terminal segment (which may or may not contain the centromere). This locus could be a "trisomy lethal" in the sense that it, in itself, has a lethal effect when present in triplicate. Alternatively, the inviability might require the combined presence in triplicate of the limiting locus and of one or more loci in the segment.

Whereas either hypothesis would adequately explain the existence of an upper limit to the length of a terminal segment for which viable partial trisomy is possible, the difference between these two hypotheses is important in another respect. If a chromosome carried one or more trisomy lethals, two or more clinically unrelated types of partial trisomy could be derived from this chromosome and there would be virtually no partial trisomics with 46 chromosomes who combine parts of two such syndromes, for their viability would require the deletion of a trisomy lethal in addition to the breaks needed for the translocation. On the other hand, if lethality of trisomy for the whole chromosome de-

pended upon a combination of two or more loci, a whole range of overlapping partial-trisomy syndromes might be found. It seems that trisomy lethals have not yet been observed in any organism, but even if such loci should not occur, say, in Drosophila this would hardly speak against their occurrence in man. Furthermore, the two hypotheses are not mutually exclusive; inviability of trisomy for a certain chromosome might be caused by a trisomy lethal, whereas in the case of other chromosomes it might be the combined effect of several loci. In the subsequent discussion either hypothesis would do equally well, and it is merely for the sake of concreteness that we shall proceed under the assumption of the existence of a trisomy lethal in the above-defined sense.

Whereas a trisomy lethal determines an upper limit for the length of the translocated segment (from a given donor chromosome) that may be present in viable partial trisomics, there is no lower limit. If the Sturge-Weber syndrome were composed of the more or less independent effects of a number of "trisomy loci"[2] scattered over this segment, one should expect the manifestation of this disease to vary much more than it does. This suggests that the major anomalies that characterize this syndrome depend on one locus or on a few loci contained in a short region of the donor chromosome. Sturge-Weber's syndrome would then be the phenotypic expression of the presence in triplicate of a terminal segment that is short enough not to contain the trisomy lethal but long enough to include the "Sturge-Weber locus" or loci. A person who is partially trisomic for a still shorter terminal segment of the same end of the "Sturge-Weber chromosome" might show none of the anomalies by which this syndrome is diagnosed.

The most difficult part in the cytological investigation of a partial-trisomy syndrome will always be the determination of the donor chromosome. As a rule, the chromosomes of an afflicted patient will not reveal its identity, but with luck it might be detected in the complement of a parent or a sib of the propositus, if that relative happens to be a carrier. Consider Fig. 5 which illustrates our interpretation of the Sturge-Weber patients investigated. Details in these diagrams are arbitrary, they represent only a few of many *a priori* possibilities. If the arm involved of the donor chromosome should be fairly large, it might be impossible to identify this chromosome in carriers related to partial trisomics with 46 chromosomes. On the other hand, carriers related to partial trisomics with 47 chromosomes offer some hope. For instance, according to our interpretation the extra chromosome in the case with 47 chromosomes could have its centromere and short arm from an acrocentric chromosome, and if this should happen to be a D chromosome, the translocation chromosome that contains most of the donor chromosome would be very conspicuous in the complement of a carrier because of the size of the attached long arm of the D (first column of the diagram in Fig. 5). In general, it seems that under almost any specific assumption about the donor chromosome one can also state a type of

[2] The term "trisomy locus" is introduced to designate a locus with a normal allele that has, generally or under certain conditions, a detectable phenotypic effect when present in triplicate in a near diploid individual.

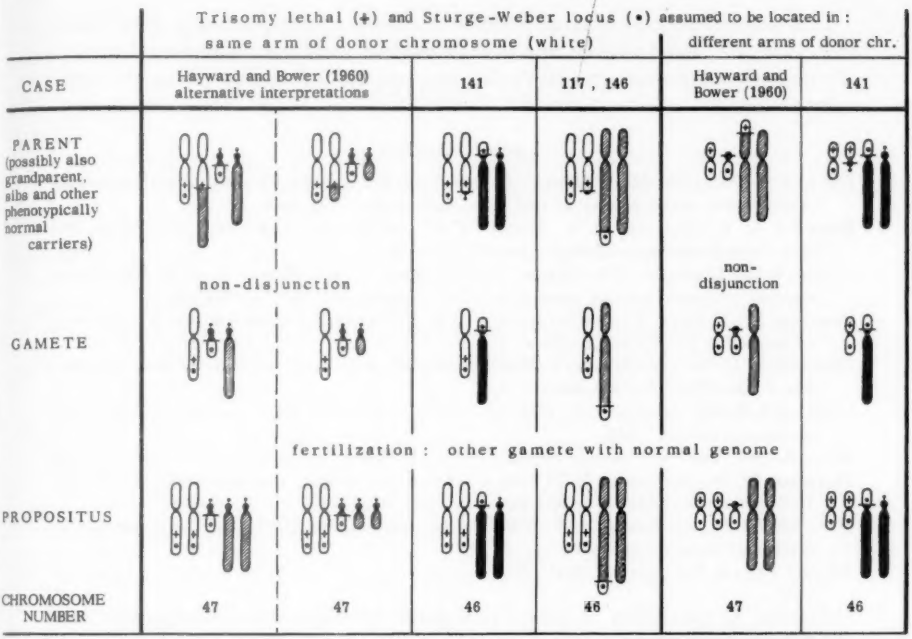

FIG. 5. Diagram illustrating several possibilities of reciprocal translocation leading to the assumed partial trisomy as cause of the Sturge-Weber syndrome. White: unknown donor chromosome; shaded: unknown recipient chromosome; black: known recipient chromosome.

translocation that, if a carrier were available, would make it possible to identify the donor chromosome or at least the group to which it belongs. Insofar as no region of any chromosome can be entirely immune to breakage we may also assume that any specific type of partial trisomic will eventually be observed. It will then be a mere matter of time until a patient of this kind is found who has a carrier among his or her parents or sibs. Thus the cytological identification of the donor chromosome, or at least its approximate characterization, appears as a problem that is in principle solvable.

SUMMARY

Three Sturge-Weber patients were found to have 46 chromosomes. The only abnormality detected was a translocation chromosome in one of these patients, a short chromosome segment from an unknown donor chromosome having evidently been attached to the short arm of a D chromosome.

It is believed that the Sturge-Weber syndrome is generally caused by partial trisomy even though the presence of a translocated extra segment, always including the same region of a certain donor chromosome, may be microscopically unrecognizable in a majority of cases. Trisomy for the whole donor chromosome may be lethal.

It is pointed out that a large number of different partial-trisomy syndromes, each rare, are bound to exist and that these may comprise a substantial part of the multiple congenital anomalies that are especially frequent among the mentally retarded.

REFERENCES

Böök, J. A., Fraccaro, M., Hagert, C. G., and Lindsten, J. 1958. Congenital malformations in children of mothers aged 42 and over. *Nature* 181: 1545–1546.

Buckton, K. E., Harnden, D. G., Baikie, A. G., and Woods, G. E. 1961. Mongolism and leukaemia in the same sibship. *Lancet* i: 171–172.

Carter, C. O., Hamerton, J. L., Polani, P. E., Gunalp, A., and Weller, S. D. V. 1960. Chromosome translocation as a cause of familial mongolism. *Lancet* ii: 678–680.

Fraccaro, M., Kaijser, K., and Lindsten, J. 1960. Chromosomal abnormalities in father and mongol child. *Lancet* i: 724–731.

Greenwald, H. M., and Koota, J. 1936. Associated facial and intracranial hemangiomas. *Am. J. Dis. Child.* 51: 868–896.

Gustavson, K.-H., and Höök, O. 1961. The chromosomal constitution of the Sturge-Weber syndrome. *Lancet* i: 559.

Hall, B. 1961. The chromosomal constitution of the Sturge-Weber syndrome. *Lancet* i: 559.

Hayward, M. D., and Bower, B. D. 1960. Chromosomal trisomy associated with the Sturge-Weber syndrome. *Lancet* ii: 844–846.

Hayward, M. D., and Bower, B. D. 1961. The chromosomal constitution of the Sturge-Weber syndrome. *Lancet* i: 558–559.

Lehman, O., and Forssman, H. 1960. Chromosomes in the Sturge-Weber syndrome. *Lancet* ii: 1450.

Michaelis, A., and Rieger, R. 1958. Cytologische und stoffwechsel-physiologische Untersuchungen am aktiven Meristem der Wurzelspitze von Vicia faba L. II. Präferentielle Verteilung der chromosomalen Bruch- und Reunionspunkte nach anaerober Quellung der Samen. *Chromosoma* 9: 514–536.

Moorhead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. M., and Hungerford, D. 1960. Chromosome preparations of leukocytes cultured from human peripheral blood. *Exp. Cell Res.* 20: 613–616.

Patau, K. 1960. The identification of individual chromosomes, especially in man. *Am. J. Human Genet.* 12: 250–276.

Patau, K. 1961. Chromosome identification and the Denver Report. *Lancet* i: 933–934.

Penrose, L. S., Ellis, J. R., and Delhanty, J. D. A. 1960. Chromosomal translocations in mongolism and in normal relatives. *Lancet* ii: 409–410.

Polani, P. E., Briggs, J. H., Ford, C. E., Clarke, C. M., and Berg, J. M. 1960. A mongol girl with 46 chromosomes. *Lancet* i: 721–724.

Therman, E., Patau, K., Smith, D. W., and DeMars, R. I. 1961. The D trisomy syndrome and XO gonadal dysgenesis in two sisters. *Am. J. Human Genet.* 13: 193–204.

The First Example of the Rh Phenotype r^Gr^G

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THE SEROLOGY OF THE Rh BLOOD GROUP SYSTEM has become too complicated to permit a simple description of new findings. Since 1958 and the description by Allen and Tippett of rh^G (G) as an antigen associated with Rh_0 (D) and/or with rh′ (C) except in the rare phenotype, r^Gr (*deG/cde*), at least nine new Rh antigenic determinants have been described. Some of these new Rh antigens have received only a "CDE" name whereas others have been given only an "Rh–Hr" name. Many are defined by only a single antiserum and the problem of establishing standards of positive and negative results has not been explored by independent typing centers.

The present report of the first example of the very rare Rh phenotype, r^Gr^G, cannot hope to clarify Rh terminology or resolve the problem of defining the variability of one Rh antigen in differing phenotypes. Nevertheless the serological properties of this rare homozygote require description because the data characterize for the first time the antigenic products of the r^G allele. For further information on the nature of some of the unusual Rh reagents employed, the reader is referred to the cited pertinent literature.

The blood of a caucasoid patient, Mr. R.B., suffering from duodenal ulcer, was submitted to the Consultation Service of the Ortho Research Foundation, because of unusual Rh typing results observed by Mrs. Boyajian (Melrose-Wakefield Hospital, Melrose, Mass.). The red cells, type O, were found to be G positive, but not positive in a standard manner for any other of the usual Rh antigenic determinants. The Rh typing reactions are summarized in table 1.

The red cells of Mr. R.B. were considered to be D negative on the basis of direct tests with several antisera tested by saline, albumin, ficin and indirect antiglobulin methods, and on the basis of absorption and elution tests. Similarly, the red cells of Mr. R.B. were found to lack E and c, and were inconsistently positive for C and e. These as well as the other Rh typing reactions to be noted in table 1, agreed with the results to be expected of r^Gr^G in accordance with the original description of r^Gr by Allen and Tippett (1958).

A family study (table 2) revealed that the deceased parents of the propositus, Mr. R.B. (II-1), had been first cousins.

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TABLE 1. RH TYPING REACTIONS OBSERVED WITH THE RED CELLS OF MR. R.B.

Strong Positive	Weak Positive	Negative
1* anti-G	10 anti-C	7 anti-D
1 anti-G eluate	6 anti-e	7 anti-E
6 anti-CD		5 anti-c
		3 anti-e
		2† anti-f
		4‡ anti-Ce
		2 anti-C^w
		1 anti-C^x
		3 anti-V

* Immune response of type rr person to type r^Gr red cells; kindly supplied by Dr. Kurt Stern, University of Illinois, Chicago.

† Including original serum (Rosenfield *et al.*, 1953).

‡ Including original serum (Rosenfield *et al.*, 1958).

TABLE 2. PEDIGREE REVEALING A PROBABLE HOMOZYGOTE OF THE Rh ALLELE, r^G

	ABO Type	Reactions with Anti-						Rh Phenotype
		C	D	E	c	e	G	
I-1, father of II-1	dead							
I-2, mother of II-1*	dead							
II-1, propositus	O	?	−	−	−	?	+	r^Gr^G
II-2, wife of II-1	A	+	+	−	+	+	+	R_1r
II-3, sister of II-1	O	+	+	−	−	+	+	R_1r^G
II-4, husband of II-3	not tested							
III-1, husband of III-2	not tested							
III-2, daughter of II-1	A	?	−	−	+	+	+	r^Gr
III-3, husband of III-4	not tested							
III-4, daughter of II-1	O	+	+	−	−	+	+	R_1r^G
III-5, daughter of II-3	A	?	−	−	+	+	+	r^Gr
IV-1, daughter of III-4	B	+	+	−	not tested			R_1
IV-2, son of III-4	B	+	+	−	not tested			R_1

* First cousin of I-1.

Mrs. R.B. (II-2), whose red cells possessed the R_1r phenotype, appears to have transmitted R^1 to one child (III-4) and r to another (III-2), while Mr. R.B. transmitted r^G to each. Child III-4, with genotype R^1r^G, could not be distinguished by direct agglutination tests from other R_1R_1 phenotypes, and a sister of Mr. R.B. (II-3) seemed to have possessed a similar R^1r^G genotype and she probably has transmitted r^G to III-5. Scores of saline agglutinating anti-D sera with R^1r^G failed to reveal a significant reduction in the expression of D as has been described for the R^1r' and R^1r^y genotypes (Levine *et al.*, 1957).

If the propositus possessed the Rh genotype r^Gr^G, his red cells afforded an opportunity to identify more completely the products of the r^G allele, and tests with three additional reagents were performed.

Anti-Hr_0 (Wiener and Wexler, 1958; Allen and Corcoran, 1958) as a triple eluate ($R_1R_1 \rightarrow R_2R_2 \rightarrow rr$) of the immune response of a person with -D- pheno-

type, was kindly provided by Dr. F. H. Allen, Jr. of Boston. The serum of immunized persons who possess the –D– phenotype have been considered to be mixtures of anti-C, anti-c, anti-E, anti-e, and anti-f, and variable cross reactions (Race *et al.*, 1950; Hackel, 1957). Successive absorption and elution from c-negative, f-negative red cells, from e-negative, f-negative red cells, and from C-negative, E-negative red cells, establishes the existence of at least one antigenic determinant that is not only independent of C, c, E, e, and f, but also which is lacking in the –D– phenotype. Thus far, only the term, Hr_0, has been employed.

Anti-Hr and anti-hr^s (Shapiro, 1960) were kindly provided by Dr. M. Shapiro of Johannesburg, South Africa. Anti-Hr is the immune response of a Negress (Mrs. Sh.) with an Rh phenotype, $\mathfrak{Rh}_0^{(v)}$ ($ccD^{u}eeffV^{u}$), that reacts with the red cells of all Rh phenotypes except –D–, $C^{w}D$– (Gunson and Donohue, 1956), and one other unrelated Negroid woman (Mrs. Sa.) who had the same Rh phenotype and was found to be similarly isoimmunized. Anti-Hr can be split by appropriate absorption and elution into a component resembling anti-e, termed anti-hr^s and anti-e: a reagent previously considered to be anti-e was shown to be a mixture of anti-e (anti-hr″) and anti-hr^s (Shapiro, 1960).

The red cells of Mr. R.B. were found to lack hr^s although they possessed both Hr_0 and Hr.

A battery of eight anti-C reagents, none of which contained antibodies directed toward D or G antigenic determinants, when tested with D + C − G + red cells of differing phenotypes, (i.e., types R_0, R_2, and –D–) were found to differ from each other. These differences did not appear to be related to the Rh phenotype of the donor of each reagent serum although, as indicated earlier by Rosenfield and Haber (1958), the anti-C from R_2R_2 persons resembles anti-rh_1 (Ce), table 3.

TABLE 3. COMPARATIVE TITERS* OF EIGHT EXAMPLES OF PURE ANTI-C REAGENTS

Serum	Phenotype of Donor	Phenotype of Test Cell			
		r′r	$r^{y}r$	$r'^{n}r$	$r^{G}_{r}G$ or r'^{G}_{r}
1. Polsby†‡	rr	128	64	32	1
2. Wilson†§	rr	128	128	8	1
3. Greene‡	R_2r	512	32	32	4
4. Woodward‖	R_2r	1024	128	256	<1
5. 2127	R_2r	1024	64	128	2
6. 1890	R_2r	128	32	32	4
7. Milwaukee¶	R_2R_2	256	2	8	4
8. Sullivan	R_2r	128	32	64	32

* Reciprocal titration values against ficin treated red cells, the method yielding the most sensitive results.

† Absorbed with D + C − erythrocytes until exhausted of anti-D + G.

‡ Kindly provided by Dr. F. H. Allen, Jr., Blood Grouping Laboratory, Boston.

§ Kindly provided by Dr. J. M. Staveley, Central Laboratory, Auckland Hospital, New Zealand.

‖ Kindly provided by Dr. T. E. Cleghorn, South London Transfusion Centre, England.

¶ Kindly provided by Dr. T. J. Greenwalt, Milwaukee Blood Center, Milwaukee.

TABLE 4. AGGLUTINATION OF FICIN TREATED RED CELLS OF DIFFERENT PHENOTYPES, OBSERVED WITH THE AGGLUTININS OF SULLIVAN SERUM (cf Anti-C #8, table 3)

Phenotype of Test Cell	Native Serum	Serum Absorbed Exhaustively with r^Gr^G or r^Gr	Eluates from r^Gr^G or r^Gr
r^Gr^G (II-1)	+	−	+
r^Gr (III-2 and Crosby*)	+	−	+
$r'r$	+	+	+
$r'r'$	+	+	+
r'^nr	+	+	+
r_yr	+	+	+
r_yr''	+	+	+
R_1r	+	+	+
R_1^wr	+	+	+
R_2r	+	+	+
R_2r	−	−	−
R_0	−	−	−
$r''r$	−	−	−
rr	−	−	−

* r^Gr propositus of Allen and Tippett (1958).

Only one of these eight sera agglutinated r^G red cells in appreciable titer, and this was the only reagent of the eight from which a positive eluate could be obtained. This eluate had the specificity of anti-C, as shown in table 4, indicating the presence of C-like antigenic determinants on r^G red cells.

All eight of these reagents could be absorbed to exhaustion of that component of their anti-C antibodies which would agglutinate r^G red cells. These absorptions always left residual anti-C antibodies of unchanged titer, but without activity toward r^G cells. Thus, if anti-C exists in two forms, ordinary anti-C and anti-C^G, with only the latter agglutinating r^G cells, the Sullivan serum (No. 8, table 3) appears to be unusually rich in the anti-C^G component.

Attempts to isolate anti-rh_i (Ce) from these anti-C reagents failed, confirming that anti-rh_i (Ce) need not occur in anti-C, but is more closely related to unusual anti-e responses. In each instance, repeated absorptions with r^yr and/or r'^nr red cells were inefficient. The red cells, r^yr and r'^nr, although C-positive lack rh_i (Ce) (Rosenfield and Haber, 1958). The term R'^sr (Race *et al.*, 1960) has also been employed to describe the r'^nr phenotype. The phenotype, $r'r$, on the other hand, readily exhausted each reagent of all antibody activity.

A battery of eight anti-e sera, as well as anti-hr^s (Shapiro, 1960) were employed in tests of the r^Gr^G cells of Mr. R.B., R^2r^G cells of a nephew of the original propositus described by Allen and Tippett (1958), the red cells of Mrs. Sh. described by Shapiro (1960), and normal R^2r red cells.

In table 5 the r^G alleles of Mr. R.B. and of the R^2r^G genotype of Allen and Tippett (1958), appear to determine e but not hr^s. This result resembles the description of the e status of Mrs. Sh. (Shapiro, 1960). Other heterogeneity in

TABLE 5. COMPARATIVE AGGLUTINATION REACTIONS OF ANTI-hr^s, AND OF EIGHT REAGENTS PREVIOUSLY CONSIDERED TO BE ANTI-e, WITH THE RED CELLS OF r^Gr^G, R_2r^G, AND MRS. SH

		r^Gr^G	R_2r^G†	Mrs. Sh.‡	R_2r
1.	anti-e	++++	++++	++++	++++
2.	anti-e	−	−	+	++++
3.	anti-e	++±	++±	++±	++++
4.	anti-e	++	++++	++±	++++
5.	anti-e	±	−	−	++++
6.	anti-e	−	±	−	++++
7.	anti-e	not done	±	±	++++
8.	AHE*	+++	+++	+++	++++
9.	anti-hr^s‡	−	−	−	++++

* Kindly supplied by Dr. F. H. Allen, Jr., Boston.

† Nephew of original r^Gr propositus described by Allen and Tippett (1958).

‡ Kindly supplied by Dr. M. Shapiro (1960), Johannesburg, South Africa.

TABLE 6. Rh ANTIGENS SEEMINGLY DETERMINED BY THE r^G ALLELE AS REVEALED IN TESTS OF THE PRESUMPTIVE HOMOZYGOTE, MR. R. B.

Positive	Negative
G, e, C^G, Hr_0*, Hr†	D, E, c, f, Ce, V, C^w, C^x, C, hr^s†

* Wiener and Wexler, 1959. Triple eluate ($R_1R_1 \rightarrow R_2R_2 \rightarrow rr$) of antibodies contained in the immune serum of a type—D—person (Allen and Corcoran, 1958); kindly provided by Dr. F. H. Allen, Jr., Boston.

† Kindly provided by Dr. M. Shapiro (1960), Johannesburg, South Africa.

anti-e reagents must exist, as suggested by the quantitative differences to be noted in table 5 with reagents number 4, 5 and 6.

In table 6 the antigenic determinants produced and not produced by the r^G allele, as determined by the observed agglutination reactions of the r^Gr^G homozygote, Mr. R.B., are summarized. The r^G allele appears to produce G, C^G, e, Hr_0, and Hr, but no other described Rh antigenic determinants.

DISCUSSION

The genotype r^Gr, described by Allen and Tippett (1958), is very uncommon, occurring in Caucasians with a frequency of perhaps one in 10,000. Additional examples have been found by Kevey *et al.* (1959) and by Stern (1960).

As the result of dosage studies, Allen and Tippett (1958) suggested that r^G determined a normal expression of G, a diminished amount of e, and little if any C or c. The allele also failed to determine D, E, f, Ce, V, C^w, and C^x.

In the present study, a blood lacking D, E, c, f, Ce, V, C^w and C^x, was found to possess only a strong expression of G, a reduced expression of e, and a minimal expression of C, consistent with the findings to be expected of the r^Gr^G homozygote. The deceased parents of this person had been first cousins, and two members of the pedigree (III-2 and III-5) possessed Rh phenotypes resembling the description of r^Gr by Allen and Tippett (1958). Two other members of the pedi-

gree (II-3 and III-4) seem to possess the R^1r^G genotype, which could not be distinguished on direct Rh agglutination tests from unselected R_1R_1 phenotypes.

The questionable C and e status of r^G gene products now seems to have been partly resolved. Anti-C occurs in at least two populations of antibody molecules, separable on the basis of their reactions with r^G test cells: anti-C^G which agglutinates and can be absorbed by r^G erythrocytes and ordinary anti-C which fails to agglutinate, or to be absorbed by r^G red cells. Thus the r^G allele appears to produce C^G but fails to produce antigenic determinants that can be classified as C. Similarly, anti-e (Shapiro, 1960) occurs in at least two populations of antibody molecules, anti-e and anti-hr^s. The r^G allele seems to determine e but not hr^s.

Anti-hr^s, because it was produced by a person who possessed the e antigen, clearly defines an antigenic determinant independent of e but resembling e in its distribution. The same cannot be said for C^G which could (and perhaps should) be interpreted with available data as a mere suppression of C that is recognized variably by different anti-C reagents.

Choice of donor blood for a patient of r^Gr^G phenotype, is not simple. In lacking both D and c antigenic determinants, r^Gr^G falls into the same risk category as r′r′ described by Levine *et al.* (1954). Furthermore, r^Gr^G also lacks ordinary C and hr^s antigenic determinants. Because of the proven antigenicity of D relative to c, donor blood of type rr would appear to be preferable.

SUMMARY AND CONCLUSIONS

1) Red cells possessing as their sole Rh antigens G, e and part of C, were encountered in the offspring of a first cousin mating in a pedigree containing two examples of r^Gr phenotypes.

2) This pattern of Rh antigenic determinants found in the red cells of the propositus is consistent with those expected of a homozygote of the r^G allele.

3) The red cells of the r^Gr^G propositus, as well as R^2r^G red cells, were found to possess e but to lack hr^s.

4) Both r^Gr^G and r^Gr were found to lack C antigenic determinants, but to possess C^G, a C-like antigenic determinant, according to eight "pure" reagents that were obtained from donors of different Rh phenotypes.

5) The r^G allele appears to determine G, C^G, e, Hr_0, and Hr, but fails to determine D, C, c, E, C^w, C^x, f, Ce, V and hr^s.

6) The genotype R^1r^G cannot be distinguished readily from R_1R_1 phenotypes.

REFERENCES

Allen, H. H., Jr. and Tippett, Patricia A. 1958. A new Rh blood type which reveals the Rh antigen G. *Vox Sang.* 3: 321–330.

Allen, F. H., Jr. and Corcoran, Patricia 1958. Evidence for new blood group antigen of nearly universal occurrence. *Proc. Am. Assoc. Blood Banks.*

Gunson, H. H. and Donohue, W. L. 1956. The blood genotype C^wD—/C^wD—. *Proc. V. Congr. Intern. Blood Trans.* 123–126. (Basel: S. Karger, 1958)

Kevey, S. V., Schmidt, P. J., and Leyshon, W. C. 1959. A second example of the blood type "rh^G". *Vox Sang.* 4: 257–266.

LEVINE, P., COOPER, MARY B., AND KOCH, ELIZABETH A. 1954. A serologic and genetic analysis of an r'r' (dCe/dCe) patient producing anti-D and anti-c. *Blood* 9: 817–823.

LEVINE, P., CELANO, M., MCGEE, R., MUSCHEL, L. H., AND GRISET, T. A. 1957. D^u and gene interaction in a family study. *P. H. Andresen Festskrift*, pp. 144–155, Copenhagen: Munksgaard.

RACE, R. R., SANGER, RUTH, AND SELWYN, J. G. 1950. A probable deletion in a human Rh chromosome. *Nature* 166: 520.

ROSENFIELD, R. E., VOGEL, P., GIBBEL, NATALIE, SANGER, RUTH, AND RACE, R. R. 1953. A new antibody, anti-f. *Brit. Med. J.* 1: 975.

ROSENFIELD, R. E., AND HABER, GLADYS 1958. An Rh blood factor rh_1 (Ce) and its relationship to hr (ce). *Am. J. Human Genet.* 10: 474–480.

SANGER, RUTH, RACE, R. R., ROSENFIELD, R. E., VOGEL, P., AND GIBBEL, NATALIE 1953. Anti-f and the "new" Rh antigen it defines. *Proc. Nat. Acad. Sci.* 30: 824–834.

SANGER, RUTH, NOADES, JEAN, TIPPETT, PATRICIA, RACE, R. R., JACK, J. A., AND CUNNINGHAM, C. A. 1960. An Rh antibody specific for V and R'^s. *Nature* 186: 171.

SHAPIRO, M. 1960. Serology and genetics of a new blood factor hr^s. *J. Forensic Med.* 7: 96–105.

STERN, K. AND BERGER, MAYA 1961. Experimental isoimmunization to rh^G. *Am. J. Clin. Path.* 35: 520–525.

WIENER, A. S. AND WEXLER, I. B. 1958. *Heredity of the Blood Groups.* New York: Grune and Stratton.

Inheritance of Human Leukocyte Antigens*

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WITH THE TECHNICAL ASSISTANCE OF MISS MILLIE TRIPP

INTRODUCTION

INTENSIVE INVESTIGATION into the immunology of both leukocytes and erythrocytes began at the end of the nineteenth century. Prior to the 1950's, however, studies on leukocyte immunology were limited for the most part to whether or not these antigens were tissue or species specific. Our knowledge of the nature of leukocyte antigens lagged far behind that of the red blood cells. The genetics and immunochemistry of erythrocyte antigens, by virtue of easily observed agglutination reactions, were more readily clarified. The summation of cumulative studies since 1900 slowly established the existence of leukocyte antigens which were clearly different from those of the platelets and red blood cells. Bedson in 1921 decisively demonstrated that an anti-guinea-pig-leukocyte serum prepared in rabbits would not agglutinate guinea pig platelets and conversely that a similarly prepared anti-platelet serum would not agglutinate the leukocytes. The presence of an antigenic difference between leukocytes and erythrocytes was confirmed by Chew, Stephens, and Lawrence (1936) who produced an anti-guinea-pig-leukocyte serum in rabbits which was free of hemolytic and hemagglutinating capacity. Working with another animal species, Amos (1954) found antigens in the leukocytes of four inbred strains of mice that were lacking in the corresponding erythrocytes.

Little material is available on differing leukocyte antigens within a single species, i.e., isoantigens. Almost all present information is confined to the isoantigens of human leukocytes. The first studies on leukocyte isoantigens attempted to detect in them the presence of A and B antigens with the aid of isohemagglutinins (Doan, 1926; Wichels and Lampe, 1928). The quest much later for naturally occurring white cell agglutinins comparable to anti-A and anti-B proved unsuccessful (Butler, 1956; Moeschlin and Schmid, 1954). It was not until 1953 that agglutinins for leukocytes were repeatedly observed in human sera (Dausset and Nenna, 1953). Leukoagglutinins were shown to be immune rather than naturally occurring isoantibodies which were capable of reacting with human leukocytes (Payne, 1957; Brittingham, 1957; van Loghem

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et al., 1957). The stimuli for their formation appeared to be either multiple transfusions or repeated pregnancies (Payne and Rolfs, 1958; van Rood *et al.*, 1959). At the present time, a number of specific leukoagglutinins which detect different leukocyte antigens may be found in immunized individuals. However, the grouping of human leukocyte antigens into systems by corresponding immune agglutinins has not been attained.

Although investigators turned their attention to the inheritance of human leukocyte antigens soon after the discovery of leukoagglutinins, this subject is still in the exploratory period. Dausset and Brecy (1957) using 28 leukoagglutinating sera compared the reaction of the leukocytes in pairs of monozygotic and dizygotic twins. The three pairs of monozygotic twins reacted similarly while the single pair of dizygotic twins did not. These findings in twins were later confirmed by Lalezari and Spaet (1959). In 1958 Payne and Rolfs demonstrated the inheritance of leukocyte antigens from the father in ten newborn infants. Six more extensive pedigrees were presented in which the family members' leukocytes had been tested with leukoagglutinins. In one family the inheritance of a specific leukocyte antigen(s) through three generations was shown. These families illustrated that the genes determining the different leukocyte antigens expressed themselves in the heterozygous state. As with classic red blood cell inheritance, an antigen present in the offspring was always present in at least one of the parents, and in no instance were antigens detected in the offspring that were lacking in both parents. Working independently in Holland, van Rood, van Leeuwen, and Eernisse (1959) reported similar results. Using nine leukoagglutinating sera, they established the incidence of corresponding leukocyte antigens in several hundred persons selected at random. The frequency of the leukocyte antigens so identified in the population varied from 14 to 85 per cent.

This paper presents the results of family studies carried out with a number of leukocyte antisera from multiparous women.

METHODS

Leukocyte antigens were identified by observing the agglutination of leukocytes with specific leukoagglutinating sera. The technique was similar to that employed with red blood cells. The method has been described in detail in a previous publication (Payne and Rolfs, 1958).

A brief resume of the procedure follows: To prepare a suspension rich in leukocytes and relatively poor in erythrocytes, defibrinated blood was mixed with a sedimenting agent (4 parts of defibrinated blood to 1 part of 4% polyvinylpyrrolidone in physiologic saline). This mixture was allowed to settle for approximately one hour after which time the supernatant containing about equal proportions of red and white blood cells was removed. This leukocyte suspension was standardized to contain about 4000 leukocytes per mm^3. If dilution was necessary, cell free serum was employed. The leukocyte suspensions were prepared daily since spontaneous agglutination develops on prolonged standing.

Antisera containing leukoagglutinins were obtained from nontransfused

women with a history of recent pregnancy. These sera were stored in the frozen state. Before use the sera were inactivated for 30 minutes at 56°C. In the test, each serum was mixed with the leukocyte suspension in the proportion of 0.15 ml. of serum to 0.05 ml. of leukocyte suspension. The mixture was then incubated for 90 minutes at 37°C. After incubation the red cells were lysed with 0.1 ml. of 3% acetic acid. The sedimented leukocytes were taken up with a Pasteur pipette, placed on a glass slide and examined microscopically at 100x magnification for agglutination. All tests were run in duplicate. Prior to testing sera with cells of incompatible ABO blood groups, the anti-A and anti-B red cell isoagglutinins were removed by absorption with group A and/or B washed red cells.

RESULTS

In the course of the investigation, antisera from 34 different women were used. Comparison of their reactions with a panel of leukocytes showed that only 19 different kinds of serum were involved. This paper is concerned with the eight sera for which more complete data were obtained.

It seems inappropriate, at this time, to propose a rigid system of terminology. This report considers a number of leukocyte antigens, each presumably defined by a single antiserum. The term "antigen" is used here in the sense of antigenic determinant, i.e., that structure or series of structures responsible for the agglutination of leukocytes by a given leukocyte antiserum. For the present, numerals will be used to distinguish among the antigens and the corresponding antisera.

Families were tested with the various antisera, and were classified on the basis of the parents' types. For purposes of analysis, the data for each type were pooled, and these are presented in table 1.

Gene frequencies were computed from the results of the reactions of the various antisera with leukocytes of unrelated individuals. In the analysis of the

TABLE 1. SUMMARY OF FAMILIES TESTED WITH EIGHT DIFFERENT LEUKOAGGLUTININS

Leukocyte Antigen	Total Families	Matings								
		Neg. × Neg.			Pos. × Neg.			Pos. × Pos.		
		Families	Offspring		Families	Offspring		Families	Offspring	
			+*	−*		+	−		+	−
1	30	5	0	11	17	28	17	8	22	4
2	26	7	0	20	17	20	20	2	5	0
3	23	2	0	3	10	19	9	11	23	1
4	19	4	0	8	8	16	7	7	11	5
5	16	2	0	8	9	12	8	5	8	0
6	24	11	0	23	11	12	13	2	8	3
10	19	1	0	1	12	15	13	6	12	0
12	17	2	0	4	7	12	2	8	14	1

* + = offspring having leukocyte antigen.
− = offspring lacking leukocyte antigen.

TABLE 2. GENE FREQUENCIES BASED ON LEUKOAGGLUTININ REACTIONS AMONG UNRELATED INDIVIDUALS

Leukocyte Antigen	Number Tested	Pos.	Neg.	p	q
1	99	62	37	.389	.611
2	94	36	58	.215	.785
3	65	46	19	.460	.540
4	66	34	32	.304	.696
5	67	34	33	.298	.702
6	54	20	34	.206	.794
10	57	39	18	.438	.562
12	52	38	14	.481	.519

data for each of the antigens under consideration the following assumptions were made: (1) the inheritance of white cell antigens is analogous to that of red cell antigens; (2) a single pair of genes is involved in the inheritance of each antigen and those individuals who are positive may be either homozygous or heterozygous for the gene producing the antigen, but those who are negative must be homozygous for the allele; (3) the population is in equilibrium; and (4) the population is panmictic. On the basis of these assumptions positive individuals comprise $p^2 + 2pq$ of the population, and the negative individuals q^2 of the population. Here p is the frequency of the gene producing the antigen under consideration and q is the frequency of the allele which does not produce the antigen. The gene frequencies obtained are shown in table 2.

The probabilities that a positive person is either homozygous or heterozygous may be obtained from the following equations: If $p^2 + 2pq$ = that portion of the population which is positive, then

$$f_{AA} = \frac{p^2}{p^2 + 2pq} = \frac{p}{1 + q},$$

and

$$f_{Aa} = \frac{2pq}{p^2 + 2pq} = \frac{2q}{1 + q}.$$

Using these probabilities, a general expression for the expected frequency of the different phenotypes among the offspring from the various types of mating may be computed. These are shown in table 3.

The comparisons of the corresponding observed and expected values are presented in table 4. There is no entry for the negative x negative mating group since no positive offspring are expected or observed for these matings (table 1). These data were subjected to the χ^2 test for goodness of fit. Since the data are not continuous, Yates' correction for continuity was applied in all cases except where it would inconsequential (Cochran, 1952). The results of this analysis are included in table 4.

In addition to these family studies, the data on incidence of the eight leukocyte antigens in unrelated individuals (table 5) were analyzed for independence (table 6). This was done by use of 2×2 tables and the resultant χ^2 values are

TABLE 3. NATURE OF OFFSPRING EXPECTED IN EACH MATING GROUP

Mating	Expected Offspring	
	Positive	Negative
Positive × Positive	$N(1 + 2q)/(1 + q)^2$	$Nq^2/(1 + q)^2$
Positive × Negative	$N/(1 + q)$	$Nq/(1 + q)$
Negative × Negative	0	N

N = Number of observed offspring in each mating group

TABLE 4. COMPARISON OF OBSERVED WITH EXPECTED OFFSPRING

Leukocyte Antigen	Mating	Positive Offspring		Negative Offspring		χ^2	P (for D.F. = 2)
		Obs.	Exp.	Obs.	Exp.		
1	Pos × Neg*	28	27.92	17	17.08	0.0006	
	Pos × Pos	22	22.26	4	3.74	0.0180	
					Total χ^2 =	0.0186	.99
2	Pos × Neg	20	22.40	20	17.60	0.3662	
	Pos × Pos†	5	4.03	0	0.97	0.2825	
					Total χ^2 =	0.6487	.73
3	Pos × Neg	19	18.19	9	9.81	0.0151	
	Pos × Pos	23	21.05	1	2.95	0.8127	
					Total χ^2 =	0.8278	.67
4	Pos × Neg	16	13.56	7	9.44	0.6762	
	Pos × Pos	11	13.30	5	2.70	1.4436	
					Total χ^2 =	2.1198	.36
5	Pos × Neg*	12	11.75	8	8.25	0.0129	
	Pos × Pos	8	6.64	0	1.36	0.6551	
					Total χ^2 =	0.6680	.71
6	Pos × Neg	12	13.94	13	11.06	0.3363	
	Pos × Pos	8	8.85	3	2.15	0.0708	
					Total χ^2 =	0.4071	.81
10	Pos × Neg	15	17.92	13	10.08	0.9078	
	Pos × Pos	12	10.44	0	1.56	0.8279	
					Total χ^2 =	1.7357	.40
12	Pos × Neg	12	9.22	2	4.78	1.6514	
	Pos × Pos	14	13.25	1	1.75	0.0404	
					Total χ^2 =	1.6918	.44

* Yates' correction for continuity not applied.

† Contains expected value of less than 1 and is included for completeness. If this is omitted, the χ^2 value for antigen 2 is 0.3662 and the value of p (for DF = 1) is .55.

shown in table 6. The underscored entries in the table are values of χ^2 which indicate non-independence at the one per cent level. The asterisks indicate those pairs in this group which according to family studies (see Appendix table) are not closely linked.

TABLE 5. FREQUENCIES OF OCCURRENCE OF TWO LEUKOCYTE ANTIGENS IN UNRELATED INDIVIDUALS

No. Tested	Leukocyte Antigen							
	1	2	3	4	5	6	10	12
11	+	+						
24	+	−						
15	−	+						
12	−	−						
19	+		+					
24	+		−					
24	−		+					
11	−		−					
12	+			+				
13	+			−				
11	−			+				
13	−			−				
22	+				+			
19	+				−			
22	−				+			
17	−				−			
20	+					+		
16	+					−		
5	−					+		
25	−					−		
8	+						+	
10	+						−	
6	−						+	
9	−						−	
13	+							+
9	+							−
11	−							+
11	−							−
30		+	+					
2		+	−					
18		−	+					
31		−	−					
11		+		+				
9		+		−				
15		−		+				
15		−		−				
12		+			+			
15		+			−			
27		−			+			
20		−			−			
6		+				+		
14		+				−		
20		−				+		
25		−				−		
28		+					+	
1		+					−	
10		−					+	
30		−					−	
15		+						+
10		+						−
18		−						+
10		−						−
12			+	+				
14			+	−				
10			−	+				
9			−	−				

TABLE 5.—*Continued*

No. Tested	Leukocyte Antigen							
	1	2	3	4	5	6	10	12
19			+		+			
13			+		−			
14			−		+			
10			−		−			
11			+			+		
25			+			−		
11			−			+		
15			−			−		
30			+				+	
7			+				−	
6			−				+	
22			−				−	
14			+					+
10			+					−
8			−					+
7			−					−
11				+	+			
13				+	−			
18				−	+			
8				−	−			
8				+		+		
15				+		−		
11				−		+		
14				−		−		
12				+			+	
13				+			−	
12				−			+	
12				−			−	
10				+				+
3				+				−
10				−				+
9				−				−
10					+	+		
20					+	−		
11					−	+		
20					−	−		
13					+		+	
19					+		−	
20					−		+	
10					−		−	
11					+			+
10					+			−
12					−			+
11					−			−
7						+	+	
16						+	−	
23						−	+	
16						−	−	
9						+		+
16						+		−
14						−		+
14						−		−
15							+	+
13							+	−
15							−	+
6							−	−

TABLE 6. SUMMARY OF INDEPENDENCE ANALYSIS OF LEUKOCYTE ANTIGENS
χ^2 VALUES OBTAINED FROM 2 × 2 TABLES

Leukocyte Antigen	1	2	3	4	5	6	10
2	2.7202						
3	3.7523	*23.7569					
4	0.0180	0.0033	0.0130				
5	0.0005	0.6999	0.0061	1.9263			
6	*8.9293	0.6770	0.4697	0.1274	0.0086		
10	0.0093	*31.9540	20.6018	0.0196	3.2366	3.6449	
12	0.0876	0.0014	0.0006	1.0450	0.0001	0.0935	0.9474

_____ = Non-independence at the 1% level
* = Not closely linked according to family studies

The Appendix table gives the phenotypes of all the families which were tested with more than one leukoagglutinin. The other families included in the study were, for a variety of reasons, tested with only one antiserum and are therefore not included in the table. With regard to genetic nomenclature, it is proposed that the symbol *W*, followed by the number of the antigen and the superscript *a*, be used for the gene producing the antigen. Thus the symbol for the gene producing antigen 1 is $W1^a$. The same symbol, with no superscript, is used for the allele or alleles which do not produce the antigen; the allele(s) of $W1^a$ is therefore *W1*. When more antigens belonging to any series are discovered, allelic genes of the series may be designated by additional superscripts, i.e., $W1^b$, $W1^c$, etc.

Several of the families included in the Appendix table show that the genes governing the production of some of the antigens are not alleles. In the Re family, the father's genotype, with regard to antigens 1 and 6 must be $W1^a/W1$ $W6^a/W6$ and the mother's must be *W1/W1 W6/W6*. Child 1, whose genotype is $W1^a/W1$ *W6/W6*, could only have received the $W1^a$ *W6* combination from the father, while her brother, whose genotype is *W1/W1 W6/W6*, must have received the *W1 W6* combination from the father. These two combinations indicate that the genes governing antigens 1 and 6 are not alleles and are not absolutely linked. A similar conclusion regarding antigens 2 and 3 may be drawn from the W family (see Appendix table). Here it is the mother who is the double-heterozygote-$W2^a/W2$ $W3^a/W3$; the father who is negative for both antigens has the genotype *W2/W2 W3/W3*. Since the father can only contribute the *W2 W3* combination, the daughter (genotype *W2/W2 W3/W3*) must have received the *W2 W3* combination from her mother, too, and the son, whose genotype is $W2/W2$ $W3^a/W3$, received the *W2* $W3^a$ combination from the mother. Hence, the genes for these two antigens are also not absolutely linked. The R_0 family shows assortment of the $W2^a/W2$ and $W10^a/W10$ genes demonstrating the lack of absolute linkage between antigens 2 and 10.

DISCUSSION

The results of these family studies show that leukocyte antigens are inherited (table 4). Their pattern of inheritance, however, is not so easily resolved. The

independence analysis indicated several pairs to be non-independent. Family studies rule out absolute linkage as the cause of non-independence on three of the cases: antigens 1 and 6 (Re family), antigens 2 and 3 (W family), and antigens 2 and 10 (Ro family). In each of these cases, as well as in the fourth case of non-independence, allelism (i.e., each of the non-independent antigens being produced by one member of an allelic pair) is not involved. If it were, the entry in the double-negative category for each pair would be zero. The presence of a sizeable number of individuals in this category (see table 5) leaves multiple antibodies or multiple alleles as the possible explanation for the non-independence. For antigens 3 and 10, absolute linkage has not been excluded, nor has the possibility that the association is due to the fact that both antigens are associated with antigen 2. The latter hypothesis is supported by the results of a simultaneous analysis of antigens 2, 3 and 10 which show that when those who are positive for antigen 2 are considered separately from those who are negative, antigens 3 and 10 are independent. (The non-independence derived earlier resulted when antigens 3 and 10 were analyzed without regard to the presence or absence of antigen 2.)

In working with leukoagglutinating antisera, a number of considerations must be kept in mind. The problem of multiple antibodies in these sera is perhaps the most serious. The likelihood of their occurrence in antisera from multitransfused patients precludes using such sera in genetic studies. Antisera produced solely as a result of immunization during pregnancy are less likely to contain as many different kinds of antibody. It should be emphasized, however, that even such sera may contain more than one kind of leukoagglutinin. Some of the evidence presented here (see tables 5 and 6) indicates that this may well be the case. In the analysis of the family data, a necessary assumption was that each of the antisera contained only one kind of antibody. While the likelihood of multiple antibodies was known to exist at the time, this had to be set aside to simplify the analysis. Evidence from this investigation as well as from other sources establishes the possibility that multiple antibodies exist in several of the antisera. For future work, these antisera will require absorption. Finally, it should be mentioned that nearly all leukoagglutinating sera tend to have low titers and therefore are used at full strength to ensure maximum reliability.

While the inheritance of leukocyte antigens is now clearly demonstrable, the questions of linkage and of allelism remain unresolved. Absorption studies now under way are expected to yield pure sera which will permit the solution of these problems. Considering the number of blood group systems in relation to the number of chromosomes, it is highly probable that some of the genes causing the production of some of the leukocyte antigens are carried on the same chromosomes as genes producing certain of the erythrocyte antigens. In the present limited investigation it has not been possible to show linkage with the ABO, MNSs, or Rh systems. However, the use of leukoagglutinins may prove to be a fruitful procedure in future work in human genetics. Leukocyte antigens considerably expand the number of hereditary traits available for study in a single sample of blood.

SUMMARY

The inheritance of human leukocyte antigens has been investigated employing eight different leukoagglutinating sera obtained from multiparous women. Population and family studies of the leukocyte antigens identified by these sera were carried out. Gene frequencies of the leukocyte antigens were calculated from tests on unrelated individuals. From these gene frequencies, the distribution of the expected offspring in various mating groups was computed and compared with those observed. The data were consistent with the hypothesis of genetic control of leukocyte antigens.

REFERENCES

Amos, D. B., Gorer, P. A., Mikulska, B. M., Billingham, R. E., and Sparrow, E. M. 1954. An antibody response to skin homografts in mice. *Brit. J. Exp. Pathol.* **35**: 203–208.

Bedson, S. P. 1921. Blood platelet anti-serum, its specificity and role in the experimental production of purpura. *J. Pathol. and Bacteriol.* **24**: 469–476.

Brittingham, T. E. 1957. Immunologic studies on leukocytes. *Vox Sang.* (N.S.) **2**: 242–248.

Butler, J. J. 1956. Some studies on the naturally occurring leukocyte agglutinins. *J. Clin. Invest.* **35**: 1150–1160.

Chew, W. B., Stephens, D. J., and Lawrence, J. S. 1936. Antileucocytic serum. *J. Immunol.* **30**: 301–318.

Cochran, W. G. 1952. The χ^2 test of goodness of fit. *Ann. of Math. Stat.* **23**: 315–345.

Dausset, J., and Brecy, H. 1957. Identical nature of the leukocyte antigens detectable in monozygotic twins by means of immune iso-leuco-agglutinins. *Nature* **180**: 1430.

Dausset, J., and Nenna, A. 1953. Présence d'une leucoagglutinine dans trois sérums de malades leucopéniques. *Le Sang.* **24**: 410–417.

Doan, C. A. 1926. The recognition of a biologic differentiation in the white blood cells. *J. Am. Med. Assoc.* **86**: 1593–1597.

Lalezari, P., and Spaet, T. H. 1959. Studies on the genetics of leukocyte antigens. *Blood* **14**: 748–758.

Loghem, J. J. van, Jr., Hart, M. van der, and Borstel, H. 1957. The occurrence of complete and incomplete white cell antibodies. *Vox Sang.* (N.S.) **2**: 257–263.

Moeschlin, S., and Schmid, E. 1954. Investigation of leukocyte agglutination in serum of compatible and incompatible blood groups. *Acta Hemat.* **11**: 241–250.

Payne, Rose 1957. Leukocyte agglutinins in human sera. *Arch. Int. Med.* **99**: 587–606.

Payne, Rose, and Rolfs, M. R. 1958. Fetomaternal leukocyte incompatibility. *J. Clin. Invest.* **37**: 1756–1763.

Rood, J. J. van, Leeuwen, A. van, and Eernisse, J. G. 1959. Leucocyte antibodies in sera of pregnant women. *Vox Sang.* (N.S.) **4**: 427–444.

Wichels, P., and Lampe, W. 1928. Die gruppenspezifische differenzierung der Leukocyten. *Klin. Wochschr.* **7**: 1741–1742.

APPENDIX TABLE

Family	Individual	Sex	Reaction with Leukoagglutinin								Erythrocyte Phenotypes		
			1	2	3	4	5	6	10	12	ABO	MN	Rh*
Reh	Fa		−	−	n	−	+	−	n	−	A	MN	R_2R_0
	Mo		−	−	n	−	−	−	n	+	A	MN	rr
	1	♂	−	−	n	−	+	−	n	+	A	N	R_0r
	2	♂	−	−	n	−	+	−	n	−	A	M	R_2r
W	Fa		+	−	−	n	n	+	n	n	A	M	R_1r
	Mo		−	+	+	n	n	−	n	n	O	M	rr
	1	♀	+	−	−	n	n	+	n	n	A	M	R_1r
	2	♂	−	−	+	n	n	−	n	n	A	M	R_1r
Ro	Fa		−	+	n	+	−	−	+	+	O	MN	R_2r
	Mo		+	−	n	−	+	+	−	+	A	N	R_1r
	1	♀	−	−	n	−	−	+	−	+	O	MN	R_1r
	2	♂	−	−	n	−	−	+	+	+	O	n	n
	3	♀	+	+	n	−	−	+	−	+	O	MN	R_1R_2
	4	♀	+	−	n	+	−	+	−	+	O	MN	R_1R_2
O	Fa		−	−	−	+	−	−	n	+	A	MN	R_2r
	Mo		−	+	+	−	−	−	n	−	O	MN	R_1r
	1	♀	−	−	−	+	−	−	n	+	A	MN	R_2r
	2	♀	−	−	−	+	−	−	n	+	O	N	rr
	3	♂	−	+	+	+	−	−	n	+	A	N	R_1R_2
	4	♂	−	+	+	−	−	−	n	+	O	M	R_1R_2
B	Fa		−	+	+	+	+	+	+	+	A	MN	R_1r
	Mo		−	+	+	+	−	−	+	+	A	MN	rr
	1	♂	−	+	+	−	+	−	+	+	A	M	rr
	2	♀	−	+	+	−	−	+	+	+	A	MN	R_1r
Re	Fa		+	+	n	−	n	+	+	+	O	MN	R_1R_1
	Mo		−	−	n	−	n	−	+	−	A	M	R_1r
	1	♀	+	+	n	−	n	−	+	−	A	MN	R_1r
	2	♂	−	+	n	−	n	−	+	+	O	MN	R_1r
Ba	Fa		+	−	+	+	−	−	+	+	A	M	R_1r
	Mo		−	+	+	+	+	−	+	+	O	MN	R_1r
	1	♀	−	+	+	+	+	−	+	+	A	MN	rr
Ba_2	Mo^1												
	Fa		+	−	+	−	+	−	+	+	O	MN	R_2R_0
	1	♀	+	−	+	+	+	−	+	+	O	MN	R_0r
	2	♂	−	+	+	+	+	−	+	+	O	MN	R_0r
Wa	Fa		−	+	n	+	n	−	+	+	O	MN	rr
	Mo		−	−	n	+	n	−	−	+	O	N	R_2r
	1	♀	−	−	n	+	n	−	−	+	O	N	rr
Wa_2	Mo^1												
	Fa		+	+	n	+	n	+	+	+	O	M	R_1R_1
	1	♂	+	−	n	+	n	+	+	+	O	MN	R_1r
	2	♀	−	+	n	+	n	−	+	+	O	MN	R_1r

APPENDIX TABLE—*Continued*

Family	Individual	Sex	Reaction with Leukoagglutinin								Erythrocyte Phenotypes		
			1	2	3	4	5	6	10	12	ABO	MN	Rh*
Ci	Fa		−	+	+	n	+	−	+	+	B	N	R_1r
	Mo		+	−	+	n	+	−	−	+	O	M	R_1r
	1	♀	−	−	+	n	+	−	−	+	B	MN	R_1r
Ci_2	Mo[1]												
	Fa		+	+	+	n	+	−	+	+	O	M	R_1r
	1	♀	−	−	−	n	+	−	−	+	O	M	R_1r
	2	♂	+	+	+	n	+	−	+	−	O	M	R_1r
Go	Fa		+	−	−	n	+	+	−	+	A	MN	R′r
	Mo		+	−	−	n	+	+	−	−	O	MN	R_1r
	1	♀	+	−	−	n	+	−	−	+	A	MN	R_1R'
Go_2	Mo[1]												
	Fa		+	−	−	n	+	+	+	−	A	M	R_1R_1
	1	♀	+	−	−	n	+	−	+	+	A	M	R_1R_1 or R_1R'
	2	♀	+	−	−	n	+	−	+	+	O	MN	R_1R_1 or R_1R'
By	Fa		+	n	n	+	n	−	−	n	O	MN	R_1R_2
	Mo[2]		+	n	n	+	n	+	+	n	A_2B	N	R_1r
	1	♀	+	n	n	−	n	−	−	n	B	MN	R_1R_1
	2	♀	+	n	n	−	n	−	+	n	B	MN	R_1R_1
Bl	Fa		+	n	−	n	n	n	n	n	A_1	N	R_2r
	Mo[2]		+	n	+	n	n	n	n	n	A_2B	N	R_1r
	1	♀	+	n	+	n	n	n	n	n	B	N	R_2r
	2	♂	+	n	−	n	n	n	n	n	A_2	N	R_1r
	3	♀	+	n	−	n	n	n	n	n	A_2	N	R_1r
	4	♂	+	n	−	n	n	n	n	n	B	N	R_2r
P	Fa		−	−	+	−	n	n	n	n	A	MN	R_1r
	Mo		−	+	+	−	n	n	n	n	O	N	R_1R_1
	1	♀	−	−	+	−	n	n	n	n	A	MN	R_1R_1
	2	♀	−	+	+	−	n	n	n	n	O	N	R_1R_1
S	Fa		+	−	n	n	n	+	−	n	A_1B	M	rr
	Mo		+	−	n	n	−	+	−	n	O	MN	R_1r
	1	♀	+	−	n	n	−	+	−	n	B	M	R_1r
	2	♀	+	−	n	n	−	+	−	n	A_1	MN	R_1r
	3	♂	+	−	n	n	−	+	−	n	B	MN	rr
	4	♀	−	−	n	n	−	−	−	n	B	M	R_1r
	5	♂	+	−	n	n	−	+	−	n	A_1	M	R_1r
	6	♂	+	−	n	n	−	+	−	n	A_1	M	R_1r
	7	♂	+	−	n	n	−	+	−	n	B	MN	rr
	8	♂	+	−	n	n	−	+	−	n	B	M	rr
	9	♂	+	−	n	n	−	+	−	n	A_1	M	R_1r
	10	♀	+	−	n	n	−	+	−	n	B	M	rr
	11	♀	−	−	n	n	−	−	−	n	A_1	MN	rr
	12	♂	+	−	n	n	−	+	−	n	A_1	MN	rr

APPENDIX TABLE—*Continued*

Family	Individual	Sex	Reaction with Leukoagglutinin								Erythrocyte Phenotypes		
			1	2	3	4	5	6	10	12	ABO	MN	Rh*
Cu	Fa		−	+	n	+	n	−	+	n	A_2	MN	R_2R_0
	Mo		+	−	n	+	n	−	−	n	O	N	R_2r
	1	♀	+	−	n	+	n	−	+	n	O	N	R_0r
	2	♀	+	−	n	−	n	−	n	n	O	N	R_2r or R_2R_0
	3	♀	+	−	n	+	n	−	−	n	A_2	N	R_2r or R_2R_0
	4	♂	+	−	n	+	n	−	−	n	O	MN	R_2R_2
	5	♂	+	+	n	+	n	−	−	n	A_2	N	R_0r
	6	♂	+	−	n	+	n	−	−	n	A_2	N	R_2r or R_2R_0
Hi	Fa		−	+	n	n	n	−	+	−	O	N	R_1r
	Mo		+	−	n	n	n	+	−	−	A_1	MN	rr
	1	♂	−	+	n	n	n	−	+	−	A_1	MN	rr
	2	♀	+	+	n	n	n	+	+	−	O	N	rr
Bs	Fa		−	+	+	n	n	−	+	−	A_1	M	R_1r
	Mo		+	−	−	n	n	+	−	+	O	MN	R_1r
	1	♂	+	+	+	n	n	+	+	+	O	MN	R_1R_1
	2	♂	−	+	+	n	n	−	+	+	O	MN	rr
Br	Fa		+	n	+	+	n	n	n	n	O	N	R′r
	Mo		−	−	+	−	+	−	+	n	A	MN	R_0r
	1	♀	n	−	+	+	+	−	−	n	A	N	R_0R'
	2	♂	n	−	+	−	+	−	−	n	O	MN	R_0R'
	3	♂	n	n	+	+	+	−	−	n	O	MN	R_0r
	4	♀	n	−	+	+	+	−	−	n	A	N	R_0R'
	5	♀	+	n	+	+	+	n	n	n	O	N	R_0r
U	Fa		−	n	+	n	n	−	n	n	B	M	R_1r
	Mo		+	n	−	n	n	+	n	n	A	N	R_1R_1
	1	♂	+	n	+	n	n	+	n	n	AB	MN	R_1R_1
	2	♀	+	n	+	n	n	−	n	n	A	MN	R_1r
	3	♂	+	n	+	n	n	−	n	n	O	MN	R_1R_1
	4	♂	n	n	+	n	n	n	n	n	A	MN	R_1R_1
Bo	Fa		+	n	−	n	n	n	n	n	O	M	R_1r
	Mo		+	n	+	n	n	n	n	n	A	M	R′r
	1	♂	+	n	+	n	n	n	n	n	O	M	R_1R'
	2	♀	+	n	+	n	n	n	n	n	A	M	rr
	3	♀	+	n	+	n	n	n	n	n	O	M	R_1r
	4	♀	−	n	+	n	n	n	n	n	O	M	R_1R'
	5	♂	−	n	+	n	n	n	n	n	O	M	R_1R'
Ja	Fa		+	−	+	n	n	−	n	n	B	N	R_0r
	Mo		+	−	−	n	n	−	n	n	A	M	R_0r
	1	♂	+	−	−	n	n	−	n	n	A	MN	R_0r
	2	♂	+	−	−	n	n	−	n	n	O	MN	R_0r

APPENDIX TABLE—*Concluded*

Family	Individual	Sex	Reaction with Leukoagglutinin								Erythrocyte Phenotypes		
			1	2	3	4	5	6	10	12	ABO	MN	Rh*
Str	Fa		+	+	n	n	−	n	n	n	O	M	R_2r
	Mo		−	−	n	n	+	n	n	n	O	M	R_0r
	1	♀	−	+	n	n	+	n	n	n	O	M	R_0r
	2	♀	−	+	n	n	+	n	n	n	O	M	R_2r
	3	♀	−	−	n	n	−	n	n	n	O	M	R_2r
	4	♀	−	+	n	n	−	n	n	n	O	M	R_2r
	5	♂	+	−	n	n	+	n	n	n	O	M	R_0r
Gi	Fa		+	−	+	n	n	+	n	n	A	M	R_1R_1
	Mo		+	−	+	n	n	−	n	n	O	M	R_2r
	1	♀	+	n	n	n	n	+	n	n	O	M	R_2R_1
	2	♀	−	−	−	n	n	+	n	n	A	M	R_2r

* = tested with anti-D, -C, -E, -c, and (when necessary) with anti-e.

[1] Child of preceding family.

[2] Mrs. By and Mrs. Bl are twin sisters.

\+ = positive with antiserum

− = negative with antiserum

n = not tested with antiserum

The Interpretation of Twin Data*

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INTRODUCTION

ONE OF THE PROBLEMS that led to the development of biometry by Galton and Pearson was the interpretation of data on physical measurements of man. In spite of this early interest in the study of quantitative attributes in man, which constitute the common and the majority of important human characteristics, human genetics has been almost totally preoccupied with rare pathologies and so-called single gene effects, (Dobzhansky, 1955; Dobzhansky and Wallace, 1954; Mather, 1953; Penrose, 1949; Snyder, 1954, 1955) effects which can be conveniently studied by traditional pedigree analysis and genealogical methods.

The pedigree methods are based on the replacement of continuous attributes by discrete attributes, e.g. PTC tasting (Harris and Kalmus, 1949), and by the assumption that the approximate discrete variable is controlled by the phases at usually a single locus or, rarely, a very few loci. This methodology has been very successful in achieving some understanding of so-called abnormalities and in general cases in which the continuous distribution has distinct modes. The type of attributes we are considering here do not fall into this category. With an attribute like height, for instance, the distribution in the population is not at all bimodal and exhibits in some respects an appearance of blending inheritance even though we are convinced that no such process happens. Similarly the treatment of environment in pedigree analysis is considered to be discrete, as, for instance, a malarial versus non-malarial environment. Undoubtedly some aspects of environment can be so classified but discontinuous or multimodal distribution is the exception rather than the rule.

In man it is the environment which must be changed to permit different genotypes with limited or particular norms of reaction to produce the desired phenotypes, (Osborne and De George, 1959a). This requires an understanding of the interaction of heredity and environment for which we must study quantitative variables employing methods other than pedigree analysis. In theory at least, an efficient method for appraising the heredity-environment problem in man, particularly with respect to complex or quantitative inheritance, is by the study of twins (Verschuer, 1939).

The twin study method began in 1875 with Galton's recognition of the usefulness of twins for evaluating the nature-nurture problem. Galton reasoned that

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the magnitude of the differences between the within-pair differences of one-egg and two-egg twins would provide an estimate of the relative importance of heredity and environment. This formulation of nature-nurture studies in twins, which is still the usual method and objective of twin studies, assumes that there are two distinct and distinguishable types of twins, monozygotic (one-egg) and dizygotic (two-egg), and that the environmental forces which affect within-pair character differences are comparable in the two types of twins.

While not yet proved, there is a possibility of a third type of twin resulting from either fertilization by different sperm cells after the division of the ovum, or from fertilization of the second polar body. If this type of twin does occur, in all probability it is a rare event. The proposition that twins occur in the two types (monozygotic and dizygotic) can be considered established. Certainly less well established is the comparability of the environmental forces which affect them. For a consideration of the methodology of twin studies it is necessary to have in mind some of the environmental factors which affect monozygotic and dizygotic twins differently.

In recent years particular interest has been taken in the prenatal environment of twins (Price 1950). Twins in general have a higher infant mortality than do the single born, and largely through application of the Weinberg differential method to twin birth and census data, it has been shown that differences in health and survival occur between different twin categories, and for twins from different definable segments of the population, (Allen, 1955; Allen and Kallmann, 1955; Lilienfeld and Pasamanick, 1955, and others). These studies have been most often interpreted as indicating that prenatal conditions are less favorable for monozygotic than for dizygotic twins, and that monozygotic twins are more affected by adverse environments. Still to be considered, however, is the fact that sex plays an extremely important role in infant mortality, and in at least some instances could affect the conclusions drawn from the use of the Weinberg differential method which is based on the unlike-sex twin pairs, (Osborne and De George, 1959a). There are in addition certain factors peculiar to dizygotic twinning which may result in unfavorable environments peculiar to dizygotic twins. Dizygotic twinning is more frequent in older women, when, on the average, conditions are less favorable for fetal development, and the incidence of congenital malformation is markedly increased. Also, in dizygotic twins, genetically conditioned competition between the fetuses can result; this is not possible in monozygotic twins, since they are genetically identical. This would be particularly pronounced when vascular communications between placentae occur.

The existence of the type of problems which has been touched upon briefly here has cast serious doubts on the meaning and reliability of heritability estimates calculated from twin data by Holzinger's formula:

$$\frac{\text{Variance of DZ} - \text{Variance of MZ}}{\text{Variance of DZ}}.$$

The initial problem for the analysis of twin data is to determine whether genetic and environmental components of variability can be measured by the

technique employed, and whether the components estimatable from various types of data are consistent with a genetic model. With attention to the biases to which twin data are subject, the methods can be extended and profitably employed under certain circumstances for studying genetic and environmental interactions (Osborne and De George, 1959a). Both the need and the possibilities exist, however, for adding considerably to the twin study method, and thereby to the most neglected areas of human genetics: the study of heredity—environment interactions and the handling of quantitative variables.

THE BASIC STATISTICAL SITUATION WITH DATA OCCURRING IN PAIRS

There are some aspects of twin and sibling data which are entirely non-genetic in nature and are common to all cases in which observations occur in pairs, as for example when we have an observation on each half of a number of leaves, or two clonal propagations of each of a number of clover plants, or two light bulbs from each of many days' production. The first point is to become clear on what may be termed the logical structure of the data and the consequences this may have on the interpretation.

We suppose that we have a (conceptual at least) population of pairs of observations, such as the heights of each of a number of twin pairs. We shall assume that the available pairs are a random sample from the population of such pairs. We assume that the pairs are not classifiable, in that, for instance, some pairs are represented at age 20, some at age 21 and so on, or, and this will more frequently be the case, that we are fairly confident that any such basis for classification does not explain total variability. We also assume that there is no basis for classification of the individuals of the pairs which runs across the pairs, in distinction to the case of siblings, for example, where we could set up a correspondence among first members of pairs by priority in birth. This assumption could lead to possible objections because of the long-honored view that classification by birth order of the twins may be important. As the importance of birth order in twins has never been demonstrated this objection will not be considered here.

One can set up a mathematical description of such a population by sampling dummy random variables, see for example Cornfield (1944) or Wilk and Kempthorne (1956), but, in a simple case like this, there appears to be little difficulty in envisaging that the two members of a pair have something in common while two individuals from different pairs have nothing in common. If we designate the pairs of observations by X_{c1}, X_{c2}, where c designates the pair, while the subscript 1 or 2 serves merely to keep track of the two distinct members, we have

$$\text{Var}\,(X_{c1}) = \text{var}\,(X_{c2}) = \sigma_x^2 \text{ (say)}$$

$$\text{cov}\,(X_{c1}, X_{c2}) = \sigma_{wx}\,.$$

This structure immediately suggests the analysis of variance given in table 1 for n pairs.

In table 1 the quantities are as follows: (a) B* is the sum of squares between

TABLE 1. ANALYSIS OF VARIANCE: OBSERVED VALUES

Source	d.f.	S.S.	M.S.
Between pairs	$n - 1$	B^*	$B = b^*/(n - 1)$
Within pairs	n	W^*	$W = W^*/n$
	$2n - 1$	T^*	

See text for explanation of symbols.

TABLE 2. ANALYSIS OF VARIANCE: EXPECTED VALUES

Source	d.f.	M.S.	E.M.S.
Between pairs	$n - 1$	B	$(\sigma_x^2 - \sigma_{wx}) + 2\sigma_{wx}$
Within pairs	n	W	$(\sigma_x^2 - \sigma_{wx})$

pairs, equal to $S_2 - C$, where S_2 is half the sum of squares of pair totals and C is the correction factor and is the square of the total of all observations divided by 2n; (b) W* is equal to T − B, and is also equal to half the sum of squares of differences of observations in pairs; (c) T* is the total sum of squares equal to $S_1 - C$, where S_1 is the sum of squares of all observations.

As is usual, the interpretation of any number is based on its expected value, and the mean squares in table 1 have expected values, or average values with an indefinitely large number of sets of n pairs, as given in table 2, and in conformity with most statistical literature these are designated as expectations of mean squares (E.M.S.). That the mean square have these expected values may be shown as follows. A typical degree of freedom of the "within pair" sum of squares is equal to

$$\frac{(X_{11} - X_{12})^2}{2}$$

which we can write as

$$\frac{[(X_{11} - \mu) - (X_{12} - \mu)]^2}{2}$$

where μ is the expected value of any X. This is equal to

$$\frac{(X_{11} - \mu)^2 + (X_{12} - \mu)^2 - 2(X_{11} - \mu)(X_{12} - \mu)}{2}$$

and we have already said that X_{11} and X_{12} cannot be differentiated, so the expected value of this is

$$E(X_{11} - \mu)^2 - E(X_{11} - \mu)\,(X_{12} - \mu)$$

where E denotes average over the population, and equals

$$\sigma_x^2 - \sigma_{wx}.$$

The quantity σ_{wx} arises from correlation of the observations within the pair resulting from likeness of genotype and environment which would tend to make

it positive, and also from competition arising from the sharing of an environment which would tend to make it non-positive.

If we now follow the intuitive procedure, which in this case appears good, of estimating unknown fixed quantities by observational quantities which have the unknowns as their expectations, we have, denoting "estimate of" by a circumflex ($\hat{}$),

$$\hat{\sigma}_x^2 - \hat{\sigma}_{wx} + 2\hat{\sigma}_{wx} = B,$$

$$\hat{\sigma}_x^2 - \hat{\sigma}_{wx} = W,$$

so that

$$\hat{\sigma}_{wx} = \frac{B - W}{2},$$

$$\sigma_x^2 = \frac{B + W}{2}.$$

Also we may consider how much of the total variability is common to the two members of each pair and this is σ_{wx}/σ_x^2 which is estimated by $(B - W)/(B + W)$. This quantity has been called "heritability" (Hancock, 1952; Stormont, 1954) under some circumstances, and in some cases there are good reasons for using this word which is likely to imply "the degree" to which a trait is inherited. It is to be emphasized, however, that this ratio has significance because of the logical structure of the data, and is of utility on this basis alone if for no other reason.

Under assumptions that are not at all unreasonable, the reliability of the estimates is indicated by their variances and their estimated variances, which will be the squares of their standard errors, as follows

Estimate	*Variance*	*Estimated Variance*
B^*	$\frac{2}{(n-1)}[E(B)]^2$	$\frac{2}{(n+1)}B^2$
W^*	$\frac{2}{n}[E(W)]^2$	$2\frac{W^2}{(n+2)}$
$\hat{\sigma}_{wx}$	$\frac{1}{2}\left\{\frac{[E(B)]^2}{n-1} + \frac{[E(W)]}{n}\right\}$	$\frac{1}{2}\left\{\frac{B^2}{n+1} + \frac{W^2}{n+2}\right\}$
$\hat{\sigma}_x^2$	$\frac{1}{2}\frac{[E(B)]^2}{n-1} + \frac{[E(W)]^2}{n}$	$\frac{1}{2}\left\{\frac{B^2}{n+1} + \frac{W^2}{n+2}\right\}$
$\frac{\hat{\sigma}_{wx}}{\sigma_x^2}$	$8\frac{B^2W^2(2n-1)}{(B+W)^4n\cdot(n-1)}$	$8\frac{B^2W^2}{\{B+W\}^4}\frac{(2n+3)}{(n+1)(n+2)}$

The estimated variance of $\hat{\sigma}_{wx}/\sigma_x^2$ is "very" approximate, if we may be permitted the use of such a term.

The Interpretation of Estimates

Roughly speaking σ_{wx} is the variability resulting from forces common to the origin of the two observations of a pair, while σ_x^2 is the variability resulting from all forces which lead to any one observation. To go further we have to consider specific situations, and in turn we take the cases of monozygotic twins, dizygotic twins, and full-sib pairs.

Monozygotic twins

A complete listing of all forces which act on both individuals of a monozygotic twin pair would be a difficult task. It is one, however, which cannot be avoided merely by saying that any listing will be too crude an approximation to be worth doing. Here is an attempted listing of common forces, which we designate by suggestive letters:

(G) The two individuals of the monozygotic twin pair have in our present state of knowledge exactly the same genetic structure, i.e. the same genes in the same order and so on.

(M) The two individuals have passed through fetal development in the same uterus.

(P) They have shared the same gross prenatal environment, i.e. they have not only shared the same uterus, but have done so at the same time, so that parity, maternal age and maternal health were constant. (In the presence of adequate obstetrical data this environmental period could be further subdivided to determine the importance of single and double chorions, etc.)

(N) The two individuals will presumably have essentially the same neonatal environment: Same hospital nursery, feeding regime, etc. (Here again, detailed data could permit further classification as to whether incubated, breast fed or artificially fed, etc.)

(I) The two members of a monozygotic twin pair share the same general environment during infancy. (Special studies, for example, of the effect of other children in the home, infectious diseases, etc. would require partitioning of this environmental force for specific effects.)

(C) The two individuals will usually share the same childhood environment. Both members are affected by the same features of the home environment. Twins raised apart, as studied by Newman, Freeman and Holzinger (1937) would require special consideration. See also Wright (1954) in this connection.

(F) The same adolescent environment will be largely shared by monozygotic twins, probably to a lesser extent by like-sexed dizygotic twins, and may be quite different for unlike-sexed pairs. This is the period when freedom of individual choice begins to be expressed. Consequently, assumptions as to shared environment become less certain.

(L) The adult environment may or may not be the same. Monozygotic twins who continue to share the same environment into adulthood should

in all probability be considered a highly selected group. It may be important to partition the adult environment in particular studies, even on as gross a level as those living together or living apart (Osborne and Adlersberg, 1958).

It is to be realized that the above forces are, in reality, shorthand statements of a multitude of forces.

In addition to considering forces which are common it is also obviously necessary, as has been emphasized by some writers on twin data, to consider competitive forces which will result in non-likeness of the two members of a monozygotic twin pair:

(Z) There may have been unequal partition of the material of the multiplied fertilized ovum. [If for instance a splitting occurred at the 4-cell stage, with a three-to-one partition, one could imagine one twin of the pair having an early advantage which was never negated.]

(U) There could have been intra-uterine competition for blood and perhaps space. [The actual study of competitive forces would require a division:
  (o) Intra-uterine competition of single-chorion pairs, which will not be present in dizygotic twins, and
  (t) intra-uterine competition of double-chorion twins which is present in dizygotic twins also, but in the presence of related G values.]

(S) Competition between individuals after birth, i.e.—differences in strength, size, general mental, and physical health.

It may be expected that the common and competitive forces have effects which vary with the genotype common to the pair, but as a first approximation we can envisage the operating forces as being independent of genotypes and additive in their effects so that we may write, with an obvious correspondence of notation:

$$\begin{aligned} X_{i1} &= G_i + M_i + P_i + N_i + I_i + C_i + F_i + L_i + Z_i + U_i + S_i + e_{i1} \\ X_{i2} &= G_i + M_i + P_i + N_i + I_i + C_i + F_i + L_i - Z_i - U_i - S_i + e_{i2} \end{aligned} \quad (1)$$

in which we have inserted a subscript i to designate the particular effect for the i-th pair, and we have added also terms e_{i1} and e_{i2} to represent contributions specific to the individuals. Competitive forces are accommodated by giving the two individuals of a pair contributions of equal size but opposite sign.

As a first approximation we also assume that the various effects are uncorrelated in the population of twin pairs, for example, that there is no correlation between G_i, the genotypic effect and M_i, the common uterine effect.

If now we define the average value of G_i over the population as μ and the average value of all other components as zero, which amounts to absorbing the nonrandom portion of each component into the nonrandom portion of the G_i component we have

$$E(X_{i1}) = E(X_{i2}) = \mu$$

$$\sigma_x^2 = \text{Var}\,(X_{i1}) = \text{Var}\,(X_{i2}) = \sigma_G^2 + \sigma_M^2 + \sigma_P^2 + \sigma_N^2 + \sigma_I^2 + \sigma_C^2 + \sigma_F^2 + \sigma_L^2 + \sigma_Z^2 + \sigma_U^2 + \sigma_S^2 + \sigma_e^2$$

$$\sigma_{wx} = \text{Cov}\,(X_{i1}, X_{i2}) = \sigma_G^2 + \sigma_M^2 + \sigma_P^2 + \sigma_N^2 + \sigma_I^2 + \sigma_C^2 + \sigma_F^2 + \sigma_L^2 - \sigma_Z^2 - \sigma_U^2 - \sigma_S^2.$$

We may note that if the twins were separated after childhood the component σ_L^2 would not occur in the covariance; if the twins were separated after infancy, the components σ_F^2 and σ_C^2 also would not be present in the covariance; and if the twins were separated at birth, the component σ_I^2 would be absent from the covariance. In this way one can visualize the data needed to separate the sources of variability. Similarly, for the competitive forces, σ_S^2 would not occur in the covariance if the twins were separated at birth, and σ_U^2 would not occur if it were biologically possible to determine whether there were two fertilized ova in the uterus and to transplant one of them to another uterus. [This could of course raise other problems of interaction of offspring genotype and maternal effects, genotypic or otherwise.] It might also be possible to assess the competition of chorionic origin mentioned under U above.

This discussion makes clear the interpretation which can be made from monozygotic twin data alone, and even a limited consideration of possible forces indicates that the ratio σ_{wx}/σ_x^2 may not give a fair estimate of the ratio of genotypic variance (σ_G^2) to the total.

Dizygotic twin pairs

If we confine ourselves to the forces listed above for the case of monozygotic twin pairs, which may be a simplification because of the possibility of interaction of the genotypes of the two members of the dizygotic twin pair, we find that

$$X_{i1} = G_i + M_i + P_i + N_i + I_i + C_i + F_i + U_i + S_i + e_{i1}$$

$$X_{i2} = G_i' + M_i + P_i + N_i + I_i + C_i + F_i - U_i - S_i + e_{i2}$$

in which it may be noted that we have

(1) eliminated the possible competitive force Z_i and

(2) taken account of the fact that the genotypes of the two members of the dizygotic twin pair will not be the same.

We now have

$$\sigma_x^2 = \sigma_G^2 + \sigma_M^2 + \sigma_P^2 + \sigma_N^2 + \sigma_I^2 + \sigma_C^2 + \sigma_F^2 + \sigma_U^2 + \sigma_S^2 + \sigma_e^2$$

$$\sigma_{wx} = \text{Cov}\,(G_i, G_i') + \sigma_M^2 + \sigma_P^2 + \sigma_N^2 + \sigma_I^2 + \sigma_C^2 + \sigma_F^2 - \sigma_U^2 - \sigma_S^2$$

The nature of cov (G_i, G_i') has been determined initially for simple genetic situations by Fisher (1918), and later, by Cockerham (1954) and Kempthorne (1954), under the assumption that the parents producing the twin pair are a random sample of the population, that there are no sex linked effects, no genetically controlled maternal effects, and no linkage (see Cockerham, 1956 for discussion of this aspect). The covariance in the case of homogamy or assortative

mating with the additional assumption of small gene effects and no epistacy was obtained by Fisher (1918). The two individuals in the dizygotic twin pair are of course full sibs genetically and the covariance, Cov (G_i , G_i') is therefore that of full sibs, designed by Cov (F.S.), and given in the case of a random mating population at equilibrium by the equation

$$\text{Cov (F.S.)} = \tfrac{1}{2}\sigma_A^2 + \tfrac{1}{4}\sigma_D^2 + \tfrac{1}{4}\sigma_{AA}^2 + \tfrac{1}{8}\sigma_{AD}^2 + \tfrac{1}{16}\sigma_{DD}^2 + \text{etc.}$$

in which σ_A^2, σ_D^2, σ_{AA}^2 etc. are components of genotypic variability. In this equation A connotes additive effects and D connotes dominance, so that σ_A^2 is the variance of additive effects, and σ_D^2 is the variance of dominance effects, σ_{AA}^2 is the variance of additive x additive effects and so on.

Sibling pairs

In this case we can write

$$X_{i1} = G_i + M_i + e_{i1} ,$$

$$X_{i2} = G_i' + M_i + e_{i2} ,$$

where we have assumed that there is no competition between the individuals of the full-sib pair, an assumption which may well be untenable (any parent must have at times speculated over what differences there would have been had his children been born in a different order). Also it may not be entirely appropriate to use the same M_i effects and a case can possibly be made for inserting N_i , I_i , C_i , F_i , and S_i .

Using these equations we have

$$\sigma_x^2 = \sigma_G^2 + \sigma_M^2 + \sigma_e^2$$

$$\sigma_{wx} = \text{Cov}\,(G_i , G_i') + \sigma_M^2$$

We may note the importance of foster-child data in the whole problem, as has been emphasized several times by Sewall Wright (1954, for example) in his interpretation of the role of heredity and environment in the formation of the I.Q.

The quantity, Cov (G_i , G_i'), which occurs for this case is the same quantity which occurred with dizygotic twin pairs.

Summary of Interpretations

It seems convenient and informative to collect together the results we have obtained so far.

Table 3 consists of the coefficients of the several components of variance in the two estimates for each case. We have inserted question marks under the terms σ_M^2, σ_C^2 and σ_F^2 for the sibling pairs, because a pair of siblings will not experience as nearly alike forces of these types as will monozygotic or dizygotic twin pairs.

From our discussion of the possible forces it will be appreciated that the three sets of data are somewhat limited in the inferences which may be drawn. One

TABLE 3. SUMMARY OF BASIS FOR INTERPRETING TWIN AND SIBLING DATA

Origin of Data	Estimate	Genotypic G	Expectation of Estimate										
			σ_M^2	σ_P^2	σ_N^2	σ_I^2	σ_C^2	σ_F^2	σ_L^2	σ_Z^2	σ_U^2	σ_S^2	σ_E^2
Monozygotic twin pairs	$\hat{\sigma}^2_{MZ}$	σ_G^2	1	1	1	1	1	1	?	1	1	1	1
	$\hat{\sigma}_{wMZ}$	σ_G^2	1	1	1	1	−1	1	?	−1	−1	−1	0
Dizygotic twin pairs	$\hat{\sigma}^2_{DZ}$	σ_G^2	1	1	1	1	1	1	?	0	1	1	1
	$\hat{\sigma}_{wDZ}$	Cov(F.S.)	1	1	1	1	−1	1	?	0	−1	−1	0
Sibling pairs	$\hat{\sigma}_S^2$	σ_G^2	1				?	?	?				1
	$\hat{\sigma}_{wS}$	Cov(F.S.)	1				?	?	?				

purpose in presenting matter in some detail is to indicate what sorts of data would enable more complete separation.

We first note some simple interpretations which are possible if the only forces are the genotype and the environmental effect specific to each individual.

Under these circumstances, and apart from sampling error, the following equalities should hold:

$$\hat{\sigma}^2_{MZ} = \hat{\sigma}^2_{DZ} = \hat{\sigma}_S^2 = \hat{\sigma}^2_{(say)} ,$$

and

$$\hat{\sigma}_{wDZ} = \sigma_{wS} ,$$

where the subscripts MZ, DZ and S refer to monozygotic twin pairs, dizygotic twin pairs, and full-sib pairs respectively. Also we would then be able to calculate the total proportion of the variability which is genetic in origin as

$$\sigma_{wMZ}/\hat{\sigma}^2$$

If we were prepared to assume no dominance and no epistacy, we would have

$$\left(\frac{\hat{\sigma}_A^2}{\sigma^2}\right) = 2\left(\frac{\hat{\sigma}_{wDZ}}{\hat{\sigma}^2}\right)$$

and also

$$= 2\frac{\hat{\sigma}_{wS}}{\hat{\sigma}^2} .$$

Taking a less restrictive case, if the post-ova stages of full-sib pairs and dizygotic twin pairs are alike we should have,

$$\hat{\sigma}_S^2 + \hat{\sigma}_{wS} = \hat{\sigma}^2_{DZ} + \hat{\sigma}_{wDZ} .$$

Statistically significant failure of this equality would indicate that this last assumption is false, or that our general model is fallacious.

It seems that under almost any circumstances we should have

$$\hat{\sigma}^2_{MZ} = \hat{\sigma}^2_{DZ} ,$$

because any contradiction of this equality (apart from sampling error) would be an indication of different competitive forces for the cases of monozygotic and dizygotic twin pairs. We should, of course, keep in mind the possibility that only fertilized eggs of particular genotypes give rise to monozygotic twin pairs.

If we can assume that the post-ova common forces are the same for monozygotic twin pairs as for sibling (separate birth) pairs, we can infer the magnitude of variability due to competitive forces in monozygotic twin pairs as

$$\hat{\sigma}^2_{MZ} - \hat{\sigma}_S{}^2,$$

and in dizygotic pairs as

$$\hat{\sigma}^2_{DZ} - \hat{\sigma}_S{}^2.$$

The Holzinger measure of heritability is in the notation of the present paper:

$$\frac{[\sigma^2_{DZ} - \sigma_{wDZ}] - [\sigma^2_{MZ} - \sigma_{wMZ}]}{[\sigma_{DZ} - \sigma_{wDZ}]}$$

(cf. Clark 1956).

Even if the only forces were purely genetic and purely environmental specific to each individual this works out from tables 1, 2, 3 to be

$$[\sigma_G{}^2 - \text{cov (F.S.)}]/[\sigma_G{}^2 - \text{cov (F.S.)} + \sigma_e{}^2].$$

What one would like in this case is $\sigma_G{}^2/(\sigma_G{}^2 + \sigma_e{}^2)$, so that even under the best possible circumstances the Holzinger heritability formula gives a very misleading picture of the role of genes and environment, unless the covariance of full sibs is nearly zero.

The Interpretation of Multiple Measurements

So far we have considered the case in which only one measurement is available for each individual of each pair. We can envisage a number of such measurements, for example, height, weight, girth and so on, and it is desirable to consider the elementary aspects of the interpretation of such data.

We shall proceed as before and first consider the case of two measurements which we designate by x_{1ij} and x_{2ij} for the i^{th} individual of the j^{th} pair of a number of pairs of individuals.

Just as we can develop an analysis of variance for one measurement, we can develop an analysis of covariance for two measurements as given in table 4.

In this table we have added subscripts to the sums of squares B and T of table

TABLE 4. ANALYSIS OF COVARIANCE OF PAIRED DATA

Source	d.f.	Sums of squares and products		
		$x_1{}^2$	x_1x_2	$x_2{}^2$
Between pairs	n − 1	B^*_{11}	B^*_{12}	B^*_{22}
Within pairs	n	W^*_{11}	W^*_{12}	W^*_{22}
Total	n	T_{11}	T_{12}	T_{22}

1 to indicate which measurement they refer to. The sums of products can be obtained by the use of the relationships

$$B^*_{12} = \tfrac{1}{2}(B^*_{1+2,1+2} - B^*_{11} - B^*_{22})$$

$$W^*_{12} = \tfrac{1}{2}(W^*_{1+2,1+2} - W^*_{11} - W^*_{22})$$

$$T_{12} = \tfrac{1}{2}(T_{1+2,1+2} - T_{11} - T_{22})$$

where $B^*_{1+2,1+2}$ for example is the sum of squares between pairs for a pseudo-attribute $x_{1ij} + x_{2ij}$. The sums of squares lead to mean squares whose nature is exactly as discussed earlier, and it remains only to explain the nature of the mean products obtained by dividing the sums of products by their respective degrees of freedom. Now, just as a typical degree of freedom of the within pair sum of squares has a sum of squares (and mean square) equal to $(x_{1i1} - x_{1i2})^2/2$ so a typical degree of freedom of the within pair sum of products has a product equal to

$$\tfrac{1}{2}(x_{1i1} - x_{1i2})\,(x_{2i1} - x_{2i2})$$

which we can write as

$$\tfrac{1}{2}[(x_{1i1} - \mu_1) - (x_{1i2} - \mu_1)]\,[(x_{2i1} - \mu_2) - (x_{2i2} - \mu_2)]$$

where μ_1 and μ_2 are the means for x_1 and x_2, which equals

$$\frac{\begin{array}{r}(x_{1i1} - \mu_1)(x_{2i1} - \mu_2) + (x_{1i2} - \mu_1)(x_{2i1} - \mu_2) \\ - (x_{1i1} - \mu_1)(x_{2i2} - \mu_2) - (x_{1i2} - \mu_1)(x_{2i1} - \mu_2)\end{array}}{2}.$$

The quantity

$$\frac{(x_{1i1} - \mu_1)(x_{2i1} - \mu_2)}{2}$$

is the product of the deviation of the x_1 attribute for the first individual of pair i from the population mean and the deviation of the x_2 attribute for the same individual from the population mean. Its expected value, like that of $\tfrac{1}{2}(x_{1i2} - \mu_1)(x_{2i2} - \mu_2)$ may be denoted by σ_{12}. To make the notation fully consistent [as might have been appropriate from the start except that the complexity looks unnecessarily detailed with one attribute] we will henceforth denote

$$\sigma^2 \text{ for } x_1 \text{ by } \sigma_{11}$$

and

$$\sigma_2 \text{ for } x_2 \text{ by } \sigma_{22}.$$

Also we note that there is no reason for inequality of

$$(x_{1i1} - \mu_1)\,(x_{2i2} - \mu_2) \quad \text{and} \quad (x_{1i2} - \mu_1)\,(x_{2i1} - \mu_2),$$

because our designation of which individual receives the final subscript '1' and which the final subscript '2' is irrelevant. The average of either term is the co-

TABLE 5. EXPECTED VALUES OF MEAN SQUARES AND PRODUCTS IN ANALYSIS OF COVARIANCE

	d.f.	Expected Mean Squares and Products		
		x_1^2	x_1x_2	x_2^2
Between	$n - 1$	$\sigma_{11} - \sigma_{w11} + 2\sigma_{w11}$	$\sigma_{12} - \sigma_{w12} + 2\sigma_{w12}$	$\sigma_{22} - \sigma_{w22} + 2\sigma_{w22}$
Within	n	$\sigma_{11} - \sigma_{w11}$	$\sigma_{12} - \sigma_{w12}$	$\sigma_{22} - \sigma_{w22}$
Total	$2n - 1$			

variance of attribute x_1 in one individual of a pair with attribute x_2 of the other individual of a pair, and we may then denote its average value by σ_{w12}, meaning the covariance of x_1 and x_2 within pairs. It follows then that the expected value of the mean product within pairs is

$$\sigma_{12} - \sigma_{w12}.$$

As regards the mean product between pairs, a similar argument shows that it is equal to

$$\sigma_{12} + \sigma_{w12} = (\sigma_{12} - \sigma_{w12}) + 2\sigma_{w12}.$$

We can therefore form table 5. The quantities in the expectations given in table 5 are components of variance and covariance, and they can be estimated by equating observed mean squares and products to expected values.

Hence for example we take

$$\sigma_{12} = \frac{B_{12} + W_{12}}{2}$$

and

$$\sigma_{w12} = \frac{B_{12} - W_{12}}{2}.$$

Formulas for precision of these estimates are at best very complex and approximate and are not given here. See, however, Reeve (1955), Robertson (1960), and Tallis (1959).

The Interpretation of Estimates

In our discussion of the interpretation of a single attribute for twin pairs we listed forces which could affect a measurement. We can envisage the same types of forces for two different attributes.

As regards genotypic effects we can envisage three sets of gene factors, say S_1, S_2, and S_3 as follows:

S_1 affects attribute 1 only,
S_2 affects attribute 2 only,
S_3 affects both attributes 1 and 2.

If S_1 and S_2 were empty sets, i.e. there were no such gene factors, then we might expect the correlation of genotypic effects to be unity. In the case of one attribute we supposed that there was no correlation between the different terms of (1). This is hardly defensible save as an approximation but we shall make the same assumption in this case also. We can then express the covariance between and within pairs in terms of components of covariance. We then get a table exactly like table 3 except that the side and top headings refer to components of covariance rather than components of variance. The same general considerations will then be found to hold as for components of variance.

OBSERVATIONAL RESULTS

To examine the theoretical constructs presented here, observed data from an earlier twin study were employed (Osborne and De George, 1959a and b). Observations obtained on the siblings of these twin pairs have been included for illustrative purposes, in spite of their limited number.

All subjects utilized in this analysis were judged to be in essentially good health on the basis of medical examinations and health histories. Blood specimens were obtained on both members of every twin and sibling pair simultaneously and determinations of their erythrocyte sedimentation rates (ESR) were performed by the same technician, employing the Westergren method. (The number of twin pairs in the ESR analysis is somewhat less than in the height and weight analyses, since ESR determinations obtained early in the period of data collection were not comparable to the later determinations, and therefore were excluded.) Stature was measured with an anthropometer to the nearest millimeter, the subject standing bare-foot; weight was taken on a hospital balance scale with the subject clothed only in a hospital examining gown. Twin zygosity determination was based upon serological studies and selected observations (Osborne and De George, 1959a).

A summary of the ESR data is given in table 6. As is well known the ESR value is more variable for females than males. There are however some marked oddities in the results:

(1) The mean square within pairs of monozygotic and dizygotic males presents a consistent picture, i.e. greater within variability for dizygotic male twins than for monozygotic male twins, the total variability σ_x^2

TABLE 6. ERYTHROCYTE SEDIMENTATION RATE DATA

Estimates	Monozygotic ♂♂	Dizygotic ♂♂	Sib ♂♂	Monozygotic ♀♀	Dizygotic ♀♀	Sib ♀♀
B*	8.55±2.64	6.39±3.42	1.35±0.60	32.79±12.86	30.17±9.10	12.14±3.24
W*	1.02± .31	2.06±1.03	6.92±2.95	1.89± .71	17.28±5.10	18.08±4.75
$\hat{\sigma}_{wx}$	3.76±1.33	2.16±1.78	−2.78±1.51	15.45± 6.44	6.44±5.21	−2.97±2.88
$\hat{\sigma}_x^2$	4.79±1.33	4.22±1.78	4.14±1.51	17.34± 6.44	23.72±5.21	15.11±2.88
$\hat{\sigma}_{wx}/\hat{\sigma}_x^2$	.79± .08	.51± .13	−.67± .21	.89± .04	.27± .20	−.20± .27
No. of pairs	21	9	6	27	22	12

being about the same for both cases as one would expect. But the male sib pairs give results which are difficult to accept in that the covariance of members of pairs turns out to be negative, and not inappreciably so. This could be the influence of maternal age for example, or difference in exposure to infection related to the within pair age differences. However, with the number of sibling pairs involved serious speculation is not justified.

(2) For the females, as for the males, the total variance σ_x^2 does not appear to be markedly different for all three types of pairs. The covariance of members of a pair decreases progressively from monozygotic twins to siblings, but one would not expect it to become negative for sibling pairs.

(3) The genotypic variability is greater in absolute magnitude for females than for males, presumably partly owing to a dependence of variance on mean.

Some observations on different sexed twins and on different sexed siblings might be revealing. The ratios of covariance of members of the pair to the variance of an individual decreases in the expected direction but the magnitudes of the changes do not appear to be in conformity with a purely autosomal genetic explanation. There is a slight suggestion of a larger ratio for monozygotic female than for monozygotic male pairs, with a considerably lower ratio for dizygotic female than for monozygotic female pairs. Part of this is possibly explainable by a higher within individual variability for females than for males and this suggests that one should have repeated measurements of this attribute for each individual, at an interval of say 6 months.

For height, a summary is given in table 7. There is a suggestion that total variability for monozygotic individuals is greater than for dizygotic individuals and it is noteworthy that variability seems to be of about the same magnitude for males and females. The degree of likeness of members of a monozygotic pair is very high and suggests almost perfect genetic determination of height. The data on this are not at all sensitive as can be seen from table 3: one would like information on twins reared apart. The degree of correlation of members of a dizygotic female pair may be less than for dizygotic males because of sampling error. It is possibly of interest to speculate on the origin of this difference if it were established beyond statistical doubt. Dizygotic male pairs will of course have the Y chromosome in common. A plausible explanation is connected with

TABLE 7. HEIGHT DATA

Estimates	Monozygotic ♂♂	Dizygotic ♂♂	Monozygotic ♀♀	Dizygotic ♀♀	Dizygotic ♂ and ♀
B*	14.04 ± 3.89	7.39 ± 3.15	15.80 ± 3.78	8.01 ± 2.12	22.17 ± 7.60
W*	0.24 ± .07	1.17 ± .48	0.20 ± .05	2.82 ± .74	17.42 ± 5.81
$\hat{\sigma}_x^2$	7.14 ± 1.95	4.28 ± 1.59	8.00 ± 1.89	5.42 ± 1.13	19.79 ± 4.78
$\hat{\sigma}_{wx}/\hat{\sigma}_x^2$	.97 ± .013	.73 ± .14	.97 ± .003	.48 ± .14	.12 ± .24
No. of pairs	25	10	34	27	16

TABLE 8. WEIGHT DATA

Estimates	Monozygotic ♂♂	Dizygotic ♂♂	Monozygotic ♀♀	Dizygotic ♀♀	Dizygotic ♂ and ♀
B*	1042.20±289.06	386.72±164.90	679.88±162.52	262.51±70.16	542.05±179.75
W*	62.26± 16.94	65.45± 26.72	40.41± 9.53	67.54±17.74	605.47±201.82
$\hat{\sigma}_{wx}$	489.97±144.78	160.63± 83.52	319.73± 81.40	97.49±36.18	−40.71±135.13
$\hat{\sigma}_x^2$	552.23±144.78	226.08± 83.52	360.15± 81.40	165.02±36.18	564.76±135.13
$\hat{\sigma}_{wx}/\hat{\sigma}_x^2$	.89± .04	.17± .15	.89± .04	.59± .12	−.07± .24
No. of pairs	25	10	34	27	16

sexual expression. It would be relevant in this connection to obtain data also on parent-offspring pairs, for both sexes of parent and both sexes of offspring. The total variability for dizygotic male-female pairs has to be interpreted with a little care: it will contain the mean of the male variance and the female variance plus half the squared difference of male and female means and this was found to fit the situation.

The data on weight (table 8) have a very similar pattern. Individual environment seems to have a more potent role, as evidenced by the ratios σ_{wx}/σ_x^2, being about 0.89 for weight compared to about 0.97 for height. The difference between male and female dizygotic pairs appears to be less in the case of weight than height: the negative value for the covariance of unlike-sexed members of a pair may be due to sampling error. The lower total variability for dizygotic pairs than monozygotic pairs again occurs. This is not of course evidence independent of the height data for the proposition that monozygotic individuals are a biased sample of all possibilities. The data for ponderal index (table 9) present a picture similar to that for height and weight.

A constantly recurring theme in all the height, weight, and ponderal index data is that the estimated values for σ_{wx} are not at all in disagreement with the hypothesis that the main cause of covariance of members of a twin pair is genetic. Of course, the precision of estimates does not allow sharper comparisons and additional data are needed. If the effects of genes were entirely additive and the only forces were the genotype of the individual (excluding, for example, the genotype of the mother) and environmental forces, one would expect that σ_{wx}

TABLE 9. PONDERAL INDEX DATA

Estimates	Monozygotic ♂♂	Dizygotic ♂♂	Monozygotic ♀♀	Dizygotic ♀♀	Dizygotic ♂ and ♀
B*	.760 ± .21	.348 ± .148	.700 ± .167	.332 ± .085	.568 ± .195
W*	.028 ± .008	.040 ± .016	.038 ± .009	.082 ± .022	.189 ± .063
$\hat{\sigma}_{wx}$	.366 ± .105	.154 ± .075	.331 ± .084	.125 ± .044	.190 ± .102
$\hat{\sigma}_x^2$	.394 ± .105	.194 ± .075	.368 ± .084	.207 ± .044	.378 ± .102
$\hat{\sigma}_{wx}/\hat{\sigma}_x^2$	.928 ± .027	.795 ± .108	.899 ± .033	.604 ± .114	.501 ± .179
No. of pairs	25	10	34	27	16

for non-monozygotic pairs would be about one-half of the value for monozygotic pairs and this seems to be approximately realized. If the only forces were additive autosomal gene effects and environmental forces peculiar to each individual the ratio σ_{wx}/σ_x^2 for dizygotic pairs must be less than half of the value for monozygotic pairs, unless there are very marked deviations from random mating. We are in the process of examining this possibility on the lines of Fisher's work (1918). This raises the further point that understanding of this picture must be based on parental values also, because one must be able to evaluate the correlation between mates. Social and racial heterogeneity in the sample will tend to induce a positive correlation because of within-group mating. To interpret the weight data on the basis of homogamy would require either a correlation in weight of mates at marriage with a strong genetic determination of weight, or a strong genetic correlation of weight with some attribute which is the basis for homogamy. Table 3 indicates that any such explanation is highly speculative because with attributes like height and weight one may expect post-natal environmental forces to have some effect.

The existence of many possible explanations with the limited data at our disposal is a part of our general theme. It is apparent that if we are to achieve understanding of the role of genetic determination of human quantitative characteristics we must obtain additional data, which could be provided by mated pairs, parent-offspring, twins reared apart, and relatives of differing degrees of

TABLE 10. CORRELATIONS AMONG ATTRIBUTES FOR TOTAL VARIATION AND TRUE PAIR VARIATION

Attributes: 1 = height, 2 = weight, 3 = ponderal index
Numbers above main diagonal refer to total variation
Numbers below main diagonal refer to true pair variation

Monozygotic Twin ♂

	1	2	3
1	1	.345	.412
2	.342	1	−.709
3	.431	−.697	1

Dizygotic Twin ♂

	1	2	3
1	1	.324	.545
2	.255	1	−.616
3	.571	−.649	1

Monozygotic Twin ♀

	1	2	3
1	1	.484	.434
2	.488	1	−.563
3	.471	−.535	1

Dizygotic Twin ♀

	1	2	3
1	1	.446	.583
2	.319	1	−.463
3	.566	−.598	1

TABLE 11. CORRELATIONS AMONG ATTRIBUTES WITH REGARD TO WITHIN-PAIR VARIABILITY

	r_{12}	r_{13}	r_{23}
Monozygotic ♂	.456	.065	−.849
Dizygotic ♂	.569	−.127	−.787
Monozygotic ♀	.506	.470	−.527
Dizygotic ♀	.599	.613	−.262

genetic relationship, e.g., half-siblings, cousin pairs. One may expect that the closer the genetic relationship, the closer will be the environmental covariance. If, of course, both changed in the same proportion, separation of genetic and environmental causation will be difficult and the most valuable data will be twins raised together and apart, siblings raised together and apart, and half-siblings.

EXAMINATION OF MULTIPLE ATTRIBUTES

We have made a partial summary of the observations by computing estimates of variance and covariance components and translating these into correlations. The results are given in table 10. We have not computed standard errors, because the amount of data is certainly too small to allow conclusions of any definiteness to be drawn. The resemblance of the tables for different types of twin pair seem remarkable. The difference between the correlation for total variability and the correlation for true pair variability is likewise small. This may be a result of the fact that most of the variability is between pairs in all cases, even though not to the same extent.

In table 11 we give the correlations among attributes as regards within-pair variability. The data indicate a difference between males and females with regard to correlation of height and ponderal index, but this difference may not be statistically significant. There appears also to be a lower correlation of weight and ponderal index for females than for males.

SUMMARY

Traditional pedigree analysis and genealogical methods, which can be used with quantitative attributes exhibiting bimodal or multimodal distributions in the population, are not effective in interpreting environmental and genetic causes of variance in the majority of human characteristics. In theory at least, twins provide an effective method for appraising the heredity-environment problem with respect to these common non-bimodal characteristics. Considered here are: a. the theoretical problems relating to the use of twin data; b. the basic statistical situation with measurement data occurring in pairs; and, c. interpretation of the statistical estimates of genetic and environmental causes of variance.

The statistical models developed are illustrated, first, by application to a physiological measurement, (erythrocyte sedimentation rate), and then to morphological measurements, (height, weight, and ponderal index). The latter are examined also by a model for multiple attributes. With these observational

data, some of the possibilities for interpreting genetic and environmental causes of variance in quantitative attributes are demonstrated. It is found that interpretation of the observational data would be extended by information concerning mating pattern, and by more definitive descriptions of the common and competitive forces affecting within pair trait variability. This information could be provided, for the greater part, by observations on mated pairs, parent and offspring, twins and siblings raised together and apart, and half siblings. The interpretation of twin data could also involve consideration of the role of genotype-environmental interaction by use of co-twin control methods, but such methods are difficult to apply to man.

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REFERENCES

ALLEN, G. 1955. Comments on the analysis of twin samples. *Acta Genet. Med. et Gemell.* 4: 143–159.

ALLEN, G., AND KALLMANN, F. 1955. Frequency and types of mental retardation in twins. *Am. J. Human Genet.* 7: 15–20.

CLARK, P. J. 1956. The heritability of certain anthropometric characters as ascertained from measurements of twins. *Am. J. Human. Genet.* 8: 49–54.

COCKERHAM, C. C. 1954. An extension of the concept of partitioning hereditary variance for analysis for covariances among relatives when epistasis is present. *Genetics* 39: 859–882.

COCKERHAM, C. C. 1956. Effects of linkage on the covariances between relatives. *Genetics* 41: 138–141.

CORNFIELD, J. 1944. On samples from finite populations. *J. Am. Stat. Assoc.* 39: 236–239.

DOBZHANSKY, T. 1955. *Evolution, Genetics, and Man.* New York: Wiley and Sons.

DOBZHANSKY, T. AND WALLACE, B. 1954. The problem of adaptive differences in human populations. *Am. J. Human Genet.* 6: 199–207.

FISHER, R. A. 1918. The correlation between relatives on the supposition of Mendelian inheritance. *Trans. Roy. Soc., Edinburgh* 52: 399–433.

HANCOCK, J. 1952. Studies in monozygotic twins. *New Zealand J. Sci. and Tech.* 34A: 131–152.

HARRIS, H. AND KALMUS, H. 1949. The measurement of taste sensitivity to phenylthiourea (P.T.C.). *Ann. Eug. Lond.* 15: 24–31.

KEMPTHORNE, O. 1954. The correlations between relatives in a random mating population. *Proc. Roy. Soc. Lond.* B, 143: 103–113.

LILIENFELD, A. M. AND PASAMANICK, B. 1955. A study of variations in the frequency of twin births by race and socio-economic status. *Am. J. Human Genet.* 7: 204–217.

MATHER, K. 1949. *Biometrical Genetics.* New York: Dover Publications.

MATHER, K. 1953. Polygenic inheritance, in: *Clinical Genetics* (ed. by A. Sorsby). St. Louis: C. V. Mosby.

NEWMAN, H. H., FREEMAN, F. N., AND HOLZINGER, K. J. 1937. *Twins: A Study of Heredity and Environment.* Chicago: University of Chicago Press.

OSBORNE, R. H. AND ADLERSBERG, D. 1958. Serum lipids in adult twins. *Science* 127: 1294.

OSBORNE, R. H. AND DE GEORGE, F. V. 1959a. *Genetic Basis of Morphological Variation.* Cambridge: Harvard University Press.

OSBORNE, R. H. AND DE GEORGE, F. V. 1959b. A genetic component of variability in erythrocyte sedimentation rate. *Am. Nat.* 93: 140–142.

PENROSE, L. S. 1949. *The Biology of Mental Defect.* London: Sigwick and Jackson.

PRICE, B. 1950. Primary biases in twin studies. *Am. J. Human Genet.* 2: 293–352.

REEVE, E. C. R. 1955. The variance of the genetic correlation coefficient. *Biometrics* 11: 357–374.

ROBERTSON, ALAN 1960. Experimental design on the measurement of heritabilities and genetic correlations. In *Biometrical Genetics*, New York: Pergamon Press.

SNYDER, L. H. 1954. Problems confronting human geneticists. *Am. J. Human Genet.* 6: 108–123.

SNYDER, L. H. 1955. Human heredity and its modern application. *Am. Sci.* 43: 391–419.

STORMONT, C. 1954. In: *Statistics and Mathematics in Biology* (ed. by O. Kempthorne and others). Ames, Iowa: Iowa State College Press.

TALLIS, G. M. 1959. Sampling errors of genetic correlation coefficients calculated from analyses of variance and covariance. *Austral. J. Stat.* 1: 35–43.

VERSCHUER, O. VON 1939. Twin research from the time of Francis Galton to the present day. *Proc. Roy. Soc., Lond.* B, 128: 62–81.

WILK, M. B. AND KEMPTHORNE, O. 1956. Some aspects of the analysis of factorial experiments in a completely randomized design. *Ann. Math. Stat.* 27: 950–985.

WRIGHT, S. 1954. The interpretation of multivariate systems. Chapter 2 of *Statistics and Mathematics in Biology*. Ames, Iowa: Iowa State College Press.

Diaphysial Aclasis (Multiple Exotoses) on Guam

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INTRODUCTION

DIAPHYSIAL ACLASIS is a hereditary disease characterized by multiple osteochondromata. The tumors appear to arise between the ages of 4 and 8, are benign and involve chiefly the axial skeleton; the ends of the long bones being the most common site of origin. The tumors grow with the child, and other skeletal abnormalities are often present, particularly bowing and shortening of the long bones. Bone cysts and enchondromata are also frequently observed in these patients. It is estimated that at least 10 per cent of affected persons develop a chondrosarcoma at the site of a tumor during their adult life. Monographs on the disease have been written by Stocks and Barrington (1925), and by Jaffe (1943).

Twenty-one cases of diaphysial aclasis were observed among the Chamorros of Guam (in the Marianas) during a period of 6 months. In this paper, we report our experience with this disease on Guam, and review some of the literature on its genetics and pathogenesis.

PRESENT DATA

A. *Frequency*

The frequency of diaphysial aclasis in Europe and North America apparently is unknown. Stocks and Barrington (1925) compiled 1189 cases from the world literature. Jaffe (1943) reported on 28 patients who had been treated at the Hospital for Joint Diseases in New York. He states that the disease, "... though not exactly common ... is by no means rare." Dr. E. B. D. Neuhauser, in a personal communication, has estimated that at the Children's Medical Center, Boston, approximately one new case per year is uncovered in the out-patient department. The out-patient department has about 90,000 annual visits.

On Guam from October, 1958 to February, 1959, 21 cases were obtained from the families of 6 propositi. All the patients were "Chamorros", a micronesian people who live in the Mariana Islands. On Guam, the principle inhabited island of the Marianas, an accurate census (Report 1959) revealed 32,000 Chamorros of all ages. The exhaustiveness of our own survey, however, is difficult to deter-

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mine. We estimate, on the basis of a survey of school children in two villages, that more than 50 per cent of the cases on Guam were discovered and place the order of the prevalence of the disease at 10^{-3}.

We are, at present, unable to infer that the disease is endemic on Guam. Amyotrophic lateral sclerosis, however, and a syndrome characterized by basal ganglion symptoms and dementia have been shown to be endemic on the Island (Kurland and Mulder 1954; Hirano, Krooth, and Kurland 1961). The etiology of these latter two diseases is not yet clear.

B. *Genetics*

1. *Pedigrees*

Pedigrees of the six propositi are given in Figs. 1 through 4. These families provided a total of 21 ascertained cases. All propositi and other persons shown as affected were x-rayed. Radiologic bone surveys were obtained on adults and adolescents, and a film of the long bones on children.

The pedigrees in Figs. 1 through 3 are consistent with autosomal dominant

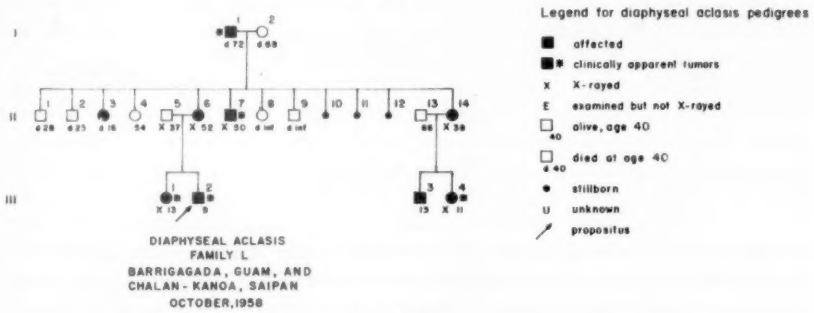

FIG. 1

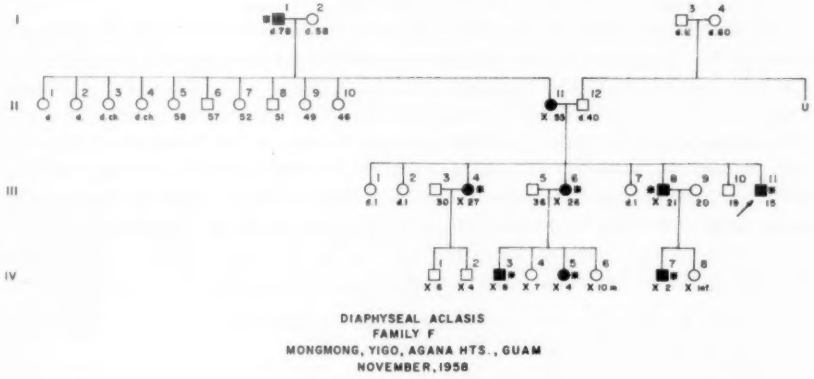

FIG. 2

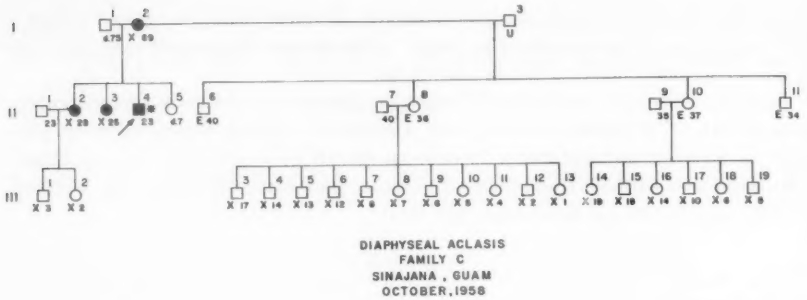

FIG. 3

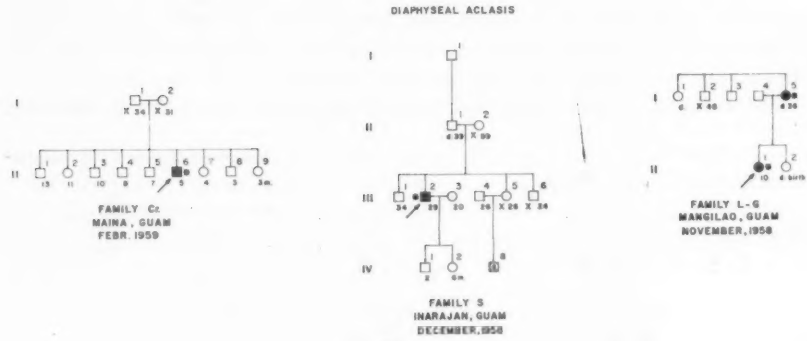

FIG. 4

heredity. The same is true of the "L-G" family in Fig. 4. The proband in the "S" family (Fig. 4) appears to be a sporadic case in the sense that the mother's bone survey was unremarkable, and no history of the father (II-1) being affected was obtained. However, for linguistic and cultural reasons, great reliability cannot be placed on purely amnestic data in this series.

The Cr family (Fig. 4) is considerably more difficult to explain on the hypothesis of simple dominance. Here both parents were examined and x-rayed, and found unaffected. The family was fairly cooperative, spoke and understood English perfectly and were well-educated. There was no *a priori* reason to question the paternity of the propositus, and social data on the family lent no support to such a possibility. Blood was drawn for typing and grouping, and although the specimens were somewhat hemolyzed in transit, data on most of the antigens were obtained. (Typing was carried out at the Blood Bank of the Clinical Center of the National Institutes of Health. We are grateful to Dr. Paul Schmidt in whose laboratory the determinations were done.) The results were:

II-1—O, MN, U^+, P^-, Cc, D, Ee, Kell(−), Fy^a, Jk^a
II-2—O, MN, U^+, P^-, C, D, e, Kell(−), Fy^a
III-5—O, MN, U^+, P^-, C, D, e, Kell(−), Fy^a

These data are therefore consistent with legitimacy. It was unfortunately not possible to obtain x-rays on other members of the family.

2. *Interpretation*

Most of the cases compiled by Stocks and Barrington (1925) were familial. The pedigrees usually showed direct transmission and no consanguinity. Both sexes were affected. Segregation occurred in many of them. It appeared, therefore, that insofar as the disease was genetic, an autosomal dominant gene might be involved. However, males were affected twice as often as females, a fact also noted by Jaffe (1943) in his series. Harris (1948) re-examined Stocks and Barrington's data and observed, among suitably informative families, that when the mother was affected the sex-ratio among affected offspring was 1:1, while when the mother was unaffected the ratio was 2:1. Finally, when the mother was unaffected, but, on the basis of her ascendancy, appeared to be transmitting the disease, the sex-ratio was 3:1.

Harris postulated that disaphysial aclasis was due primarily to an autosomal dominant gene, but that a second, independently segregating autosomal dominant could suppress the disease in the female. Using this hypothesis and the Hardy-Weinberg relations, he was able to predict the frequency of affected males and females from different classes of matings. These predictions were tested against Stocks and Barrington's (1925) data, and good agreement was obtained. It is of incidental interest that Harris estimated the frequency of the hypothetical modifying gene to be about 30 per cent, on the basis of Stocks and Barrington's (1925) data. If the gene does in fact exist, it presumably represents a genetic polymorphism. [Whether it is the Rh negative gene, *r* (c d e), or some other already recognized polymorphic gene, is of course unknown.]

Our own series appears to be consistent with Harris's (1948) suggestion, but is too small to permit us to test his theory further. Certainly the S and Cr families (Fig. 4) might be explained by such a segregating suppressor gene in the family. It is interesting to note that a number of affected adult females had no clinical signs of the disease [e.g. II-14 in family L (Fig. 1) and II-11 in family F (Fig. 2)], but were found unquestionably to be affected on x-ray examination. This was not true of any of the males who were examined. The relatively greater obesity of the adult females may have been a factor, but even on x-ray the discrepancy between the sexes in the size and number of tumors was apparent. The females were not always less severely affected, but from patient to patient seemed more variable with respect to severity than the males. The males in all cases had many large tumors.

The distribution of the patients in this series by sex of affected parent is as follows:

Parent Affected	Males	Females	Total
Mother	5	8	13
Father	2	3	5
Uncertain	2	1	3
Total	9	12	21

Although these data in themselves do not show the effect Harris described, they are small in number and, except for the total sex-ratio, do not differ significantly from the ratios in Harris's (1948) tables. It is of interest that 5 of the 6 propositi were males (Figs. 1 to 4), again indicating that the male is more apt to be conspicuously affected. Perhaps, it is rather rare for the disease to be so completely suppressed in the female that even x-ray studies disclose no involvement. The Cr family is the only example where this appears a likely possibility.

3. *Transmission of specific tumors*

In general Stocks and Barrington (1925) found no correlation between relatives for site of involvement. There were two exceptions, however. A significant correlation was observed between parent and child for involvement at a few uncommon sites. Secondly, in occasional instances when both parent and child were unilaterally affected at the site of some specific bone there was a significant tendency for both to be affected on the same side.

Our data are too sparse for such an analysis, but they show the absence of a striking correlation for site of involvement, or, comparing adults, for size of tumor at a single site. Figure 5 contains x-rays of members of the L family, the member being identified by his pedigree number. Although the x-rays are not technically perfect, they give some idea of the variation within families of severity of involvement at different sites. Note the pelvic films of the two affected adult sisters II-6 and II-14 (Fig. 5).

C. *Clinical features*

1. *Complaint and history*

The chief complaint of most of the patients in our series was cosmetic, the tumors tending to interrupt the normal contours of the extremities. The proposi-

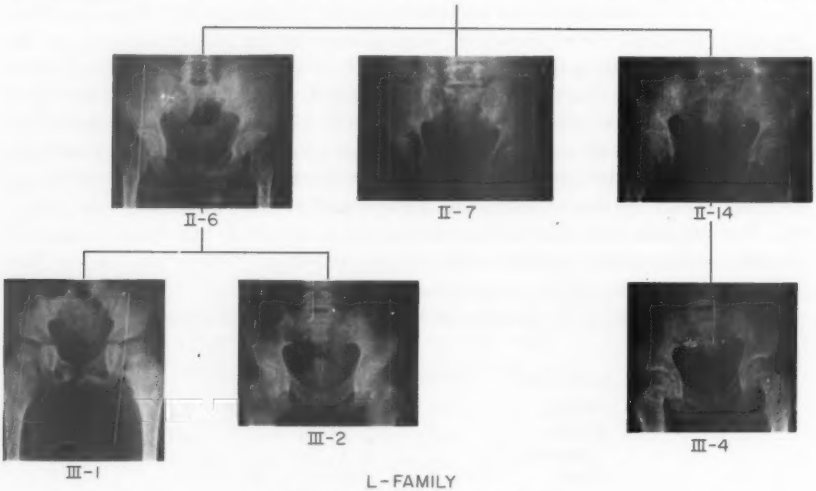

FIG. 5. Pelvic films of the L family.

tus of the S-family sought medical aid when a tumor of the left knee so restricted the joint that the patient was barely able to walk. Patient II-6 in the L family gave a history of dystocia, which is readily understood in view of her pelvic film (Fig. 5). Since the survey was done, one patient not in our present series, was discovered at autopsy to have a metastatic chondrosarcoma.

2. *Physical findings*

In all of the males the tumors were evident on inspection and this was true in half of the females. Tumors were most readily palpable at the wrist, elbow, and clavicle. They could be felt in all males, and six females.

3. *Laboratory examination*

Extensive laboratory determinations were not performed on our patients. Bloods from several patients revealed a normal serum calcium, phosphorous, and alkaline phosphatase. Todd *et al.* (1959 & 1961) have done urinary calcium and phosphorous studies on several patients from the Continental United States and these too were normal. These authors have suggested that the phosphate diuresis in response to injected parathormone may be reduced in patients with diaphysial aclasis. Urine chromatography, kindly performed on specimens from 5 of our patients by Dr. H. Eldon Sutton, then of the University of Michigan, showed no differences when compared with a series of control specimens.

PATHOGENESIS OF DIAPHYSIAL ACLASIS

The classical theories of Keith and Virchow on the pathogenesis of diaphysial aclasis have been critically reviewed by Jaffe (1943). The precise sequence of events which produces the tumors is unknown. Certainly, these osteochondromata differ from most benign tumors in their early age-at-onset. Interestingly, the same is true of solitary osteochondroma (Price, 1958).

Recently Lorincz (1961) has reported that patients with diaphysial aclasis have a mucopolysaccharidurıa. The mucopolysaccharide excreted has been identified as chondroitin sulfuric acid-A. It may therefore eventually be found that patients with this disease have an inherited abnormality either in the synthesis or metabolism of ground substance.

It has also been shown (Ponseti, 1954; Yeager and Hamre, 1957) that young rats fed extracts of the sweet pea (*Lathyrus odoratus*) develop multiple osteochondromata. Such extracts, depending upon the experimental conditions, can induce a variety of connective tissue lesions (Wazonek *et al.*, 1955; Pentschew, 1958; *inter alia*), including dissecting aneurysms, arthritic changes, etc. The effect of the extract can be duplicated by aminoacetonitrile or beta amino proprionitrile (Schilling and Strong, 1954).

The osteochondromata are probably not identical with the human ones. Their histology is somewhat different, for they may contain hematopoetic elements. Moreover, they arise primarily at the sites of muscular attachments to the bone. Hamre and Yeager (1958) have shown that the development of a tumor at a particular site can be prevented by section of the nerve to the muscle inserting at the site.

TABLE 1. SOME GENETIC TUMORS OF MAN

Disease	Genetics
Multiple Neurofibromatosis	Dominant
Xeroderma pigmentosa	Recessive
Adenoma Sebaceum (Epiloia)	Dominant
Diaphysial Aclasis	Dominant
Multiple Polyposis of the Colon	Dominant
Retinoblastoma	Dominant
Lipomatosis	Dominant
Multiple Cutaneous Leiomyomata	Dominant

These findings are of interest primarily because they suggest that a connective tissue poison may induce osteochondromata. In both the human disease and the experimental one, however, it is a little difficult to see how an abnormality in ground substance, assuming this is indeed the primary factor, can lead to a *neoplasm*. In this respect, the nerve section experiments described above are particularly illuminating.

Diaphysial aclasis probably belongs to the general group of inherited abnormalities of mesenchyme, first compiled by McKusick (1956). In virtue of its neoplastic character, however, it may also belong to a second smaller group of diseases. Suppose one makes a list of all the familial human neoplasms one can think of which have the following properties:

1. The disease appears to be due to a single gene.
2. Nearly everyone who has the gene gets the neoplasm.
3. Nearly everyone who has the neoplasm has the gene.

A list of such neoplasms is given in table 1. If one now compares these tumors with neoplastic diseases in general, several features characterizing the former group become evident. First, with the exception of retinoblastoma, which may not satisfy the third requirement listed above, these tumors are benign, at least initially. Secondly the tumors seem to arise from multiple, apparently independent, foci of origin. In the case of retinoblastoma, it has been suggested (Bell, 1922; Best, 1934; Weller, 1941; Franceschetti and Bischler, 1946; Falls and Neel, 1951) that the binocular form is more common among familial than among sporadic cases.

None of these diseases has been shown to involve abnormalities in supporting or connective tissue. They do, however, have a similaritv to diaphysial aclasis.

SUMMARY

Twenty-one cases of diaphysial aclasis, noted on the Island of Guam, are reported, and the genetic and clinical features of the disease are discussed.

REFERENCES

BELL, J. 1922. Glioma retinae. *Treasury of Human Inheritance* 2: 112.

BEST, F. 1934. Uber Vererbung des Netz-hautglioms; Bemerkungen zu den Mendelschen Regeln. *Klin. Mbl. Augenh.* 93: 209.

FALLS, H. F. AND NEEL, J. V. 1951. Genetics of retinoblastoma. *A.M.A. Arch. Ophthalmol.* 46: 367.

FRANCESCHETTI, A. AND BISCHLER, V. 1946. Retinoblastoma et heredité. *Arch. Julius Klaus-Stift.* 21: 322.

HAMRE, C. J. AND YEAGER, V. L. 1958. Influence of denervated muscles on exostoses of rats fed a sweet pea diet. *A.M.A. Arch. Path.* 65: 215.

HARRIS, H. 1948. A sex limiting modifying gene in diaphysial aclasis. *Ann. Eugen. Lond.* 14: 165.

HIRANO, A., KROOTH, R. S., AND KURLAND, L. T. 1961. Parkinsonism-dementia complex, an endemic disease on the Island of Guam. *Brain* (In press).

JAFFE, H. L. 1943. Hereditary multiple exostoses. *Arch. Path.* 36: 335.

KURLAND, L. T. AND MULDER, D. W. 1954. Epidemiologic investigations of amyotrophic lateral sclerosis. *Neurology* 4: 355 & 438.

LORINCZ, A. E. 1961. Heritable disorders of acid mucopolysaccharide metabolism in humans and in snorter dwarf cattle. *Genetic Disorders in Disease Resistance and Susceptibility. Proc. New York Acad. Sci.* (In press).

MCKUSICK, V. 1956. *Heritable Disorders of Connective Tissue.* St. Louis: C. V. Mosby Company.

PENTSCHEW, A. 1958. Intoxikationen (Lathyrismus pp. 2341–2349) in Lubarsch, O., Rossle, R., and Henke, R. (editors). *Handbuch der Spezielen Pathologischen Anatomie und Histologie.* 13: part 2. Berlin: Springer-Verlag.

PONSETI, I. V. 1954. Lesions of the skeleton and other mesenchymal tissues in rats fed sweet pea (Lathyrus odoratus) seeds. *J. Bone Surg.* 36: (Series A) 1031.

PRICE, C. H. G. 1958. Primary bone forming tumors in man and their relationship to skeletal growth. *J. Bone Surg.* 40: (Series B) 574.

REPORT 1959. Census of the Territory of Guam. *Municipal Commissioners Report.* Unpublished.

SCHILLING, E. D. AND STRONG, F. M. 1954. Isolation, structure, and synthesis of a lathyrus factor from lathyrus odoratus. *J. Am. Chem. Soc.* 76: 2848.

STOCKS, P. AND BARRINGTON, A. 1925. Hereditary disorders of bone development. *Treasury of Human Inheritance.* 3: part 1.

TODD, J., NICKERSON, F. AND HILL, S. R. 1959. Pseudo-pseudohypoparathyroidism and hereditary multiple exostosis. *Am. J. Med.* 27: 327.

TODD, J. N., HILL, S. R., JR., NICKERSON, J. F. AND TINGLEY, J. O. 1961. Hereditary multiple exostosis, pseudo-pseudohypoparathyroidism, and other genetic defects of bone, calcium, and phosphorous metabolism. *Am. J. Med.* 30: 289–298.

WAZONEK, S., PONSETI, I. V., SHEPARD, R. S., AND WIEDENMAN, L. G. 1955. Epiphyseal plate lesions, degenerative arthritis and dissecting aneurism of rat aorta produced by aminoacetonitrile. *Science* 121: 63.

WELLER, C. V. 1941. The inheritance of retinoblastoma and its relationship to practical eugenics. *Cancer Research.* 1: 517.

YEAGER, V. L. AND HAMRE, C. J. 1957. Histology of lathyrus-induced exostoses of rats: The initial change at tendon-bone junction. *AMA Arch. Path.* 64: 171.

BOOK REVIEWS

Principles of Human Genetics

By Curt Stern. San Francisco: W. H. Freeman and Co., 1960, 753 pp., $9.50.

The first edition of *Principles of Human Genetics* became a classic in its field. The rapid advances that have occurred in the field since its appearance in 1949, though not altering the principles, have (inevitably) left the text a little outmoded in terms of factual material. The rewritten and somewhat expanded second edition of Professor Stern's book reestablishes its preeminence as a basic textbook of human genetics. As the author states in the Preface, the book is designed to serve many masters, and indeed it does.

The first ten chapters are intended for all readers. The germ cells, fertilization, mitosis, meiosis, the human chromosome complement and its variations, gene action, probability and the rules of single factor inheritance, are covered briefly but adequately. The special problems of establishing genetic ratios in human families are well presented and the sib and *a priori* methods of correcting ratios from pooled data are developed by the use of simple algebraic procedures. More sophisticated approaches to this knotty problem are, wisely, omitted. The final chapter of the first section develops the Hardy-Weinberg Law from first principles, and demonstrates its applications to problems such as the breakdown of isolates, the presence of duplicate factors, and the recent intermixture of populations.

Among the subsequent chapters, the author states, are some that will interest some groups of readers more than others, but they can all be read with profit by those who wish to acquire a solid grounding in the principles of human genetics. The treatment of the many facets of modern human genetics, including developments in cytogenetics and the problems of mutation, is thorough and remarkably up-to-date. The book (quite rightly) does not attempt to catalogue the inheritance of human traits, normal or pathological, except where they illustrate principles or have some particularly interesting feature. For all that, Professor Stern has managed to incorporate a surprising amount of specific information in his discussion of principles, and does not hesitate to use examples from experimental organisms when this serves the purpose better.

The lay-out of the volume is similar to that of the previous edition, with the addition of many useful tables, figures and illustrations. Each chapter is followed by a number of problems, to test the student's understanding, and a list of selected references to the important sources of information discussed in the chapter and to further suggested readings in the field. In addition, the headings for tables and figures often contain references to their sources, providing further guides to the original literature.

The book contains more material than would be considered necessary for most introductory courses in human genetics but is so designed that the beginning student can read it with profit, omitting the more advanced sections of each chapter. Thus it is also suitable for a more advanced undergraduate course in human genetics, and can also be read with profit by students at the graduate level. Because of its size and the amount of detail it contains it will probably not be recommended for most medical school courses in genetics, in their present regrettably cachectic state, but with the rapidly growing interest in the field it may not be too long before this limitation will disappear. In the

meantime it would be nice if Professor Stern would provide us with a condensed version directed more specifically to the needs of the medical student.

Genetic counselors will find a chapter on this subject early in the book, and also sections in later chapters that interpret the significance of particular subjects to counseling, and there is an excellent discussion of the non-statistical personal and medical aspects of counseling at the end of the chapter on medical genetics. Anthropologists will benefit particularly from the chapters on race, heredity and environment, mutation, and selection. Those involved in medical research on problems with a genetic aspect will have a special interest in the chapters on genic action, lethal genes, variations in gene expression, prenatal interactions, heredity and environment and medical genetics. Psychologists will find a well-balanced discussion of intelligence and other mental traits. Geneticists will find able expositions and interesting new facts throughout the book.

In short, this is an excellent, thorough and up-to-date basic textbook of human genetics. As one with personal experience of the difficulty in keeping up with the rapid expansion in this field, this reviewer can say, with feeling; "I don't see how he does it, but I'm glad he does!".

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Human Races

By Stanley M. Garn. Springfield: Charles C Thomas, 1961, 137 pp. $5.50.

It is not often that a book can be about equally useful to beginning students and to specialists in a given branch of science. The short book under review accomplishes just this. It is written simply and clearly, and takes for granted only most elementary biology and little else. At the same time it contains, briefly stated, some generalizations and original ideas which one would not commonly expect in a book aimed at a general reader. The book is a successor to, and in a sense a considerably changed, and improved, second edition of the "Races", by C. S. Coon, S. M. Garn, and J. B. Birdsell (1950).

The first two chapters tell what races are and what they are not. "A race ... is a *population*, a population of men, women and children, of fathers, mothers, and grandparents.... Members of such a breeding population share a common history, and a common locale. They have been exposed to common dangers, and they are the product of a common environment. For these reasons, and especially with advancing time, members of a race have a common genetic heritage". Races are neither linguistic, nor religious groups, nor nations, nor hypothetical primordial "types", nor living nor fossil individuals, nor arbitrary groupings of such individuals. It would be useful to add that different races are *genetically different populations.*

Two further chapters give a review of the morphological, physiological, and sociological differences between races. Most races are geographically separate (allopatric), but in man some races occur in the same territory, sympatrically. The interbreeding of sympatric races is limited or prevented by linguistic, religious, economic or other cultural barriers. It is not desirable to refer to the cultural barriers as reproductive isolating mechanisms, which are genetic, not cultural, barriers. Perhaps *cultural isolating mechanisms* may be a useful designation. The fact that race differences are not confined to external body shapes but extend to physiological and biochemical traits is properly empha-

sized. I do not, however, think it entirely fair to say that those who attempted to use serological criteria for race classification "... had confused their criteria (here the blood groups) with the races they were trying to describe". Most of the serologists possessed a degree of sophistication in genetics which prevented them from making so crude a mistake!

Four chapters (V–VIII) are devoted to natural selection and to genetic drift as moulders of the race differences. Ten years ago, when Coon, Garn and Birdsell made suggestions concerning the possible adaptive significance of racial differences in man, these suggestions were almost pure speculations. It is gratifying to find that many observations have been made since then, giving substance to the speculations, and that these observations are concisely summarized in the book under review. This is not to deny for a moment that the existing information is still woefully inadequate, and that collection of further data is imperative. The two following chapters (IX and X) give brief and sensible discussions of such "fighting" problems as race mixture and race intelligence. Race mixture is viewed as neither intrinsically dangerous nor yielding desirable results, such as heterosis. "So far, there is no evidence for racial differences in character and temperament, other than those due to cultural conditioning." On the other hand, "Racial differences in gene-determined response patterns are very likely, especially when one considers the adaptive nature some of these response patterns must hold in nature. Racial differences in behavior therefore, may fall to the psycho-physiologists to discover, and will not encompass most areas of social behavior". And: "A very reasonable guess is that races are comparable in the sum and total of what we call 'intelligence', but differ in many interesting details".

The concluding chapter (XI) gives a classification of human races, which differs appreciably from that given in Coon, Garn, and Birdsell's book. Garn prefers a two-level classification, starting with 9 "geographical races", subdivided into "local races". The nine races are Amerindian, Polynesian, Micronesian, Melanesian-Papuan, Australian, Asiatic, Indian, European, and African. A "selected list" of 32 "local races" are really subdivisions of the Amerindian, Australian, Asiatic, Indian, European, and African "geographical races". Those who would prefer not to make the distinction between the "geographical" and the "local" races (a tenuous distinction at best, as Dr. Garn himself would probably admit), might simply add the Polynesian, Micronesian, and the Melanesian-Papuan to the 32 others, and thus emerge with 35 "races" without qualifying adjectives.

Garn's book is published at an opportune time. Although race prejudice arises from misdirected emotion rather than from reasoning, racism has time and again tried to shore itself up with quasi-science or pseudo-science. It is hardly surprising that, given the current political and social crisis, renewed attempts are being made to misuse genetics to support racism. On the opposite flank, some well-meaning authors are attempting to counteract racism by specious arguments pretending that the human species is not composed of races at all. The effect of this is the opposite of what these authors intend; the racists are given an opportunity to score an easy victory; human races are no more or no less real than are races of many animal and plant species. Reading the book of Garn should be the minimal requirement for any one who desires to discuss the problem of race rationally.

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Comparative Effects of Radiation

Edited by MILTON BURTON, J. S. KIRBY-SMITH AND JOHN L. MAGEE. New York: John Wiley and Sons, Inc., 1960, 426 pp., $8.50.

THIS BOOK is the result of a symposium on the "Comparative Effects of Radiation" held at the University of Puerto Rico in February, 1960. It was one of four symposia held within a year which dealt with aspects of the disposition of absorbed energy in biological material. This volume contains articles concerned with the molecular processes involved in the initial excitation and energy transfer; with some of the factors which determine radiochemical reactions; and with the detailed analysis of several photobiological effects. This book, of course, does not have the editorial polish of a textbook, neither does it have the unity of one. The participants of the symposium disagreed on many points. However, it is precisely those honest and unresolved disagreements which constitute one of the chief values of the volume by documenting—as of 1960—some of the important research questions in this many-faceted thing called radiobiology.

The basic theory and some experimental details concerning the processes of excitation and energy transfer are examined from a variety of viewpoints by Fano, Kasha, Magee, Förster, and Kallman and in an outstanding discussion session led by Platt. Both the student and the researcher who is concerned with these processes will find clear expositions of some of the most crucial problems by top experts. These papers clearly show some of the limitations of the present theories as they might be applied to biological systems, and in so doing set forth some of the theoretical problems which are of concern to radiobiology in the immediate future.

The other papers deal with the sequences of events which lead from the initial physical interaction to the final biological changes. Swanson documents that various types of radiation act through different mechanisms. His presentation serves as a warning not to hope for a simple unified scheme applicable to all radiobiological phenomena. The Pullmans discuss the relation between the radiosensitivity of the constituents of nucleic acids and their electronic structure. The production and distribution of chemical intermediates are discussed by Hochanadel and Hamill, their effects on macromolecules by Charlesby, and their possible biological significance by Ebert. The possible role of monolayers in radiobiological effects is discussed by Hutchinson.

The situation with visible light is somewhat simpler than with ionizing radiation since at least the absorption process is better defined. In this field some remarkably complete analyses are given by Hendricks (phytochromes) and Rupert (photoreactivation).

A major purpose of the symposium was to establish contact between workers in physics, chemistry and biology. This is a difficult task, and one that requires much continued effort. This is illustrated by the fact that the two reviewers felt that neither of them alone could do justice to the entire book. Accordingly, it is felt that few readers will be able to profit from all of the chapters, but also that almost everyone will profit from some.

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The Evolution of Man: a Brief Introduction to Physical Anthropology

By GABRIEL W. LASKER. New York: Holt, Rinehart and Winston, 1961, xvi + 239 pp., $3.50.

FEW HUMAN GENETICISTS are aware of how much their specialty is taught in American colleges. Parts of it reach over 2,000 students in Los Angeles alone. Under the rubric of "human evolution" or elaborations of the title "anthropology," some genetic knowledge is disseminated in hundreds of junior colleges, four-year colleges, and universities. Only a handful of professional geneticists, other biologists, or physical anthropologists actually teach this subject. Instead, the job is mainly in the hands of instructors trained in the humanities or in the behavioral, social or earth sciences. These people are often out of contact with professional evolutionists and geneticists, and are in great need of better textbooks and teaching aids in this field.

The preceding information comes from a nation-wide survey by Gabriel W. Lasker, editor of *Human Biology* and one of the ablest and most public-spirited physical anthropologists in America. Before undertaking this survey, he wrote *The Evolution of Man*. His pithy and convenient little book fills this need for text materials very neatly indeed.

At the moment, courses in human evolution seem to be among the most widespread vehicles for creating an informed and favorable attitude toward human genetics among American college students. If a course literally followed the outline of Lasker's book, about a quarter of it would be devoted to matters such as the origin of life, the replication of DNA, evolutionary rates among ancient and modern human populations, elementary Mendelism, serology, and the polymorphisms of such loci as ABO, Rh, the sickling and thalassemia loci, and primaquine sensitivity.

Lasker's book has enough clarity and simplicity so that an instructor with a conscience about genetics could deviate from it considerably in organizing his lectures toward some of the most timely themes in the study of heredity in man. Some of these topics might include the mutagenic effects of ionizing radiation, gene-flow models of Negro-White hybridization in the United States, heredity counseling and eugenics, and ecological factors in some of man's known genetic polymorphisms.

The brevity of this volume sometimes forces Lasker into stylistic awkwardness. He retains more traditional generic names of fossil men than most recent authors, and on page 49 errs in calling the Hominidae a "subfamily." His chapters on heredity and race, however, are superb. Let no one doubt that Lasker has written a succinct, conservative, and authoritative account of human evolution.

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Principles of Genetics

By ELDON J. GARDNER. New York-London: John Wiley and Sons, Inc., 1960, 366 pp., $7.50.

IN THE PAST TWO YEARS at least three texts have appeared with titles identical to already established genetic treatises (Inborn Errors of Metabolism, Hsia; Human Heredity, Montague: and now Principles of Genetics, Gardner). Though this phenomenon must

reflect to some degree the rapid growth and increasing popularity of our field, it is difficult to believe that the number of genetic texts has increased to the point where original titles are no longer possible; this book bears the title of a classic in the field.

Professor Gardner has written a well organized and comprehensive book which should find wide acceptance as an elementary text in genetics. The student is introduced to the subject by the historical approach, beginning with a general survey of pertinent biological information and history, and followed by a careful review of Mendel's experiments. Preparation for advanced topics is then achieved by devoting a chapter to physical mechanisms involved in cellular heredity, following which the book rapidly expands to the more complex and specialized aspects of genetics. The book is well illustrated and the drawings of important geneticists throughout the text adds an enhancing touch. A good selection of references and problems are given at the end of every chapter.

In attempting to be all inclusive, Professor Gardner has been forced to give short shrift to several subjects (e.g. genetic fine structure, genetic control of protein synthesis). Considering the length of the book, this of course is unavoidable, but since it is the more complex phases that are shortchanged this may cause teaching difficulties. While the book is not intended as a human genetics text, it will be of interest to human geneticists to know that examples of human heredity are used as illustrative material where ever possible.

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Books Received

Human Heredity

By JEAN ROSTAND. New York: Philosophical Library, 1961, 139 pp., $4.75.

A translation of a book originally published in French under the title L'Hérédité Humaine. This is a popular book written for non-specialists in the field of genetics.

Radioactive Substances

By MARIE CURIE. New York: Philosophical Library, 1961, 94 pp., $2.75.

A translation from the French of the thesis presented to the Faculty of Sciences in Paris by Madame Curie.

Genetic Research

By ARNE MÜNTZGING. Stockholm: LTs Forlag, 1961, 345 pp.

A translation of a book which was first published in Swedish in 1953. The contents have been brought up to date, so that this may be considered a second edition. It is a textbook in general genetics and attempts to cover the entire field in its fundamental and applied aspects.

Advances in Blood Grouping

By ALEXANDER S. WIENER. New York: Grune and Stratton, 1961, xii + 549 pp., $11.00.

This volume is a collection of reprints of papers by Wiener and his colleagues published since 1954. The papers have been arranged by topic in logical order, and comments have been added where needed for the sake of continuity and to bring the subject up to date.